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Aim and Scope

Operative Dentistry publishes articles that advance the practice of operative dentistry. The scope of the journal includes conservation and restoration of teeth; the scientific foundation of operative dental therapy; dental materials; dental education; and the social, political, and economic aspects of dental practice. Review papers, book reviews, letters and classified ads for faculty positions are also published.

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We thank all our reviewers for their time and dedication to Operative Dentistry.

On The Cover

"Gasteracantha versicolor in the Ranomafana National Park" Ranomafana, Madagascar. Photo provided by Kevin Matis of Indianapolis, IN USA. Photo taken with a Nikon D5100, 50mm f/5.6 1/60 sec. ISO-720. © Operative Dentistry, Inc.

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Guest Editorial

Dr. Susanne T. Grennell

Being a long-time member of the Academy of Operative Dentistry has been a fantastic experience for me. The annual meeting and the Journal have advanced my knowledge, and people I've met have inspired me and encouraged me to aim higher. The atmosphere at the annual meeting is collegial and friendly. All in all, the Academy of Operative Dentistry is a forum for learning and growing in excellence in dentistry.

I've had the opportunity to serve the Academy of Operative Dentistry in varying capacities, as president, on committees, and, currently, as chair of the Founders' Fund. The Founders' Fund was established specifically to enhance the art and science of operative dentistry by providing financial support for programs in education and research. This is for those of us in every arena: for those in private practice, schools, public services, and the military; for students as well as degree-holding dentists.

The Founders' Fund has underwritten a number of causes over the years. Currently, we fund the Ralph Phillips Student Research Award and the Rising Star Award. We have money in secure certificates of deposit that has been raised through your generous donations. According to our bylaws, we are directed to use only the interest generated by the principle to further our mission. For the past number of years, interest levels have been low, and we have received fewer donations. This has left us unable to fund as many projects as we would like. The Ralph Phillips and Rising Star Awards currently consume all our spendable funds. Fortunately, we still have a small but eroding cushion of accumulated interest available. Because of this, the Founders' Fund was able to cover a request made last summer when a newly graduated dental resident wanted to come to our

annual meeting. His finances were limited, making it impossible for him to cover the registration. As we all know, education has become a high-ticket expense. We all appreciate the dilemma young graduates have in trying to establish their lives while contending with serious debt. When young dentists are interested in excellence in operative dentistry, the Trustees of the Founders' Fund sincerely want to encourage them to become members of the Academy of Operative Dentistry. Setting up another award to support meeting attendance for a young dentist, or perhaps a senior dental student, merits our attention. Yet, no matter how worthy this may be, the limited resources currently within the Founders' Fund make consideration of this idea difficult or impossible.

Excellence in dentistry thrives within the Academy of Operative Dentistry. For encouraging excellence in dentistry and interest in membership in the Academy, I am asking all our members to contribute to the Founders' Fund. Please give online or use the brochure. Remember—this donation is fully tax deductible. Along with your donation, you may choose to honor or contribute to the memory of someone.

The honored individual will receive a notification from the Academy. This is an ideal way to show appreciation for someone special as well as appreciation of our Academy.

Let us keep our Academy strong. Thank you for your generous support.

Note: 2019 Founders' Fund contributors will be recognized on a list in the 2020 Annual Meeting Program.

Use of Transparent Tips for Obtaining Tight Proximal Contacts in Direct Class II Composite Resin Restorations

V Alonso • M Caserio • IL Darriba

Clinical Relevance

The use of the LM-Contact Former system, which uses transparent plastic cone-shaped tips, allows obtaining tight proximal contacts in posterior Class II composite resin restorations with a simple technique. Filling the remaining cavity after removing the transparent tip with a bulk-fill flowable resin-based composite decreases the total working time.

SUMMARY

This article describes the clinical technique of using the transparent plastic tips of the LM-Contact Former system for obtaining tight proximal contacts in direct Class II composite resin restorations using noncontoured circumferential matrix bands.

With this technique, the composite resin is pressed with the tip, which adapts intimately to the walls of the proximal box. Moreover, the total working time is reduced by filling the

cavity, which remains after removing the transparent tip, with a bulk-fill flowable resin-based composite.

INTRODUCTION

The area of the proximal contact in posterior teeth is located in the middle third or at the junction of the occlusal third with the middle third of the proximal surfaces.¹ Obtaining tight and anatomical proximal contacts in direct Class II composite resin restorations helps maintain the interdental papilla and prevent food impaction, periodontal disease, tooth movement, and caries.^{2,3} The interproximal contact is suggested to be contoured as an area buccolingually centered below the marginal ridges. The proximal surface contour should be very convex in the middle and occlusal thirds and flat or concave in the cervical third. Also, the interproximal tooth contacts maintain dental arch stability by transmitting forces along the long axis of the teeth.⁴

Currently, most clinicians consider that tighter proximal contacts are achieved by using sectional matrix bands in conjunction with separation rings,

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Table 1: *List of Materials Used*

Material	Manufacturer
Rubber dam	Hygenic Dental Dam, Coltène/Whaledent, OH, USA
Wedge with a metal fin	FenderWedge Directa AB, Upplands Väsby, Sweden
Caries-detector dye	Caries Detector, Kuraray, Tokyo, Japan
Gingival margin trimmer	Margin Trimmer MT26, Hu-Friedy Mfg Co, Chicago, IL, USA
Cylindrical bur that cuts only on the flat end	Komet 108, Brasseler GmbH & Co, Lemgo, Germany
Circumferential matrix band	Hawe Steel matrice, Kerr, Orange, CA, USA
Matrix system	Hawe SuperMat, Kerr, Orange, CA, USA
Wooden wedge	Hawe Sycamore Interdental Wedges, Kerr, Orange, CA, USA
LM-Contact Former	LMDental, Parainen, Finland
LM-MultiHolder PK II	LMDental, Parainen, Finland
Adhesive system	Prime & Bond XP, Dentsply Detrey GmbH, Konstanz, Germany
Light-curing unit	Demi Plus, Kerr, Orange, CA, USA
Microhybrid resin composite	Herculite XRV Enamel Unidose refill, Kerr, Orange, CA, USA
Bulk-fill flowable resin-based composite	SDR, Dentsply Detrey GmbH, Konstanz, Germany
Diamond-metal finishing strips	Diamond Finishing Strips cxds3-superfine, Edenta AG, Hauptstrasse Switzerland
Polishing disc	Polishing disc 12.7 DF-M, AXIS Dental, Crissier, Switzerland

rather than using circumferential matrix band systems.⁵⁻⁸ However, this clinical technique is complex and is not useful when the proximal box extends onto buccal or lingual walls.⁹

Because it is difficult to contour a composite resin against the contact areas of adjacent teeth when circumferential matrix bands are used, some special instruments have been developed to apply pressure to the contact area during light curing.¹⁰ These include transparent cone-shaped tips (LM-Contact Former, LMDental, Parainen, Finland), special hand instruments, Contact Pro (CEJ Dental, San Juan Capistrano, CA, USA), PerForm (Garrison Dental, Uebach-Palenberg, Germany), and restoration inserts (beta quartz glass-ceramic inserts). The transparent plastic tips are inserted into the proximal box using pressure. Consequently, the composite resin is pushed against the walls of the cavity, and the matrix band is pressed against the proximal surface of the adjacent tooth.

In addition, the technique used to place the composite resin influences the achievement of anatomical proximal contacts. With the centripetal technique, the proximal wall is formed first, placing the composite resin on the inner surface of the matrix band from gingival to occlusal, and the cavity is then filled similarly to a Class I restoration. This technique minimizes the C-factor, the polymerization shrinkage, and the formation of marginal gaps.¹¹

Another problem in the performance of composite restorations is polymerization shrinkage, which can

cause marginal failure, discoloration, secondary caries, fractures, and postoperative sensitivity.¹² It is important to note that light curing at a distance can reduce mechanical properties and affect long-term durability of the composite restorations.^{13,14} The efficacy of polymerization also significantly decreases with increasing cavity depth.^{15,16}

Recently, bulk-fill flowable resin-based composites have been introduced, which can be placed in 4-mm-thick increments. This reduces the working time without increasing polymerization shrinkage, cavity adaptation, or the degree of conversion.¹⁷ Researchers have shown clinical results similar to those obtained with incremental filling techniques.^{18,19}

The aim of this article is to describe the clinical technique of using the transparent tips of the LM-Contact Former system in direct Class II composite resin restorations using noncontoured circumferential matrix bands.

CLINICAL TECHNIQUE

The materials used are listed in Table 1. The isolation of the operative field with a rubber dam must be extensive.²⁰ In distal caries, the clamp should be placed, if possible, on two teeth behind the tooth to be restored, to prevent the wings of the clamp from interfering with the proper placement of the wedge and/or the matrix band. If there are no posterior teeth, the use of a wingless clamp is indicated. The authors always isolate to the central incisor of the same side. Using heavy-gauge rubber dams is preferred because they may be stretched



Figure 1. Available sizes of the transparent tips of the LM-Contact Formers system (LMDental, Parainen, Finland).

without tearing and therefore may be passed easier through the interproximal spaces.

Prior to cavity preparation, a thick wedge with a metal fin (FenderWedge, Directa AB, Upplands Väsby, Sweden) is inserted into the interproximal space with curved mosquito forceps to separate and protect the adjacent tooth.⁶

For the removal of the dentin caries, a round tungsten bur with a diameter corresponding to the cavity size is used at low speed. Although caries-detector dye (Caries Detector, Kuraray, Tokyo, Japan) has some limitations, the operator uses it as a guide for removing the infected dentin.²¹ When the caries removal leaves unsupported enamel at the gingival wall, it is necessary to use a gingival margin trimmer²² (Margin Trimmer MT26, Hu-Friedy Mfg Co, Chicago, IL, USA) or a cylindrical bur that cuts on only the flat end (Komet 108, Brasseler GmbH & Co, Lemgo, Germany) to remove this thin enamel.

Once the cavity preparation is finished, the protective wedge is removed and a matrix band is placed to perform the restoration. A 0.03-mm-thick metallic noncontoured circumferential matrix band (Hawe Steel Matrices, Kerr, Orange, CA, USA) is used, adapted with the Hawe SuperMat system (Kerr).

Subsequently, a wooden wedge (Hawe Sycamore Interdental Wedges, Kerr) is inserted with mosquito forceps, applying the necessary force to adapt the matrix band closely to the gingival contour of the cavity. It must also remain apical to the gingival margin, because if the wedge remains above, it will cause a convexity of the matrix band toward the cavity. The wedge also achieves separation between the teeth to compensate for the thickness of the matrix band.

Table 2: LM-Contact Former Tips		
Size	Length, mm	Diameter in the Widest Occlusal Area, mm
XS	5.30	2.30
S	4.5	2.6
M	6.0	3.3
L	6.8	4.3

Then, the largest possible transparent tip of the LM-Contact Former (LMDental, Parainen, Finland) that can be introduced into the cavity is selected (Figure 1; Table 2). The LM-MultiHolder PK II (LMDental, Parainen, Finland) is used to transport the tip and verify that the chosen size is adjusted to the cavity. When inserting the tip, some pressure must be applied so that the wider part is at the level of the proximal contact, and as a consequence, the matrix band will be adapted to the adjacent tooth. This is essential for obtaining an optimal location of the contact area, an anatomic proximal contour, and a healthy environment for the gingival papilla.

Once the adequate tip is selected, the cavity is acid etched. The adhesive (Prime & Bond XP, Dentsply Dentsply Detrey GmbH, Konstanz, Germany) is applied according to the manufacturer's instructions, and it is polymerized for 20 seconds with a light-curing unit (Demi Plus lamp, Kerr).

A layer of micro-hybrid composite resin (Herculite XRV Enamel Unidose refill, Kerr) is then placed at the bottom of the cavity, and the selected transparent tip is inserted with the LM-MultiHolder exerting pressure; thus, the composite resin is condensed against the cavity walls and the matrix band. The excess material in the occlusal area that prevents the tip from removal is removed, and the composite resin is light cured for 40 seconds. Then, the tip is removed with mosquito forceps, and the composite resin is light cured for another 20 seconds.

At that point, it may be observed that the composite resin is adapted to the cavity walls, except in the future proximal contact area, due to the pressure exerted by the transparent tip on the matrix band (Figures 2 and 3). If the cavity is a three-surface Class II, one of the proximal boxes is restored first, followed by the other box (Figure 4).

Once the tip is removed, the remaining cavity is filled with a bulk-fill flowable resin-based composite (SDR, Dentsply Detrey GmbH), except for the final occlusal layer. This is restored with the micro-hybrid composite resin (Herculite XRV Enamel Unidose refill, Kerr).

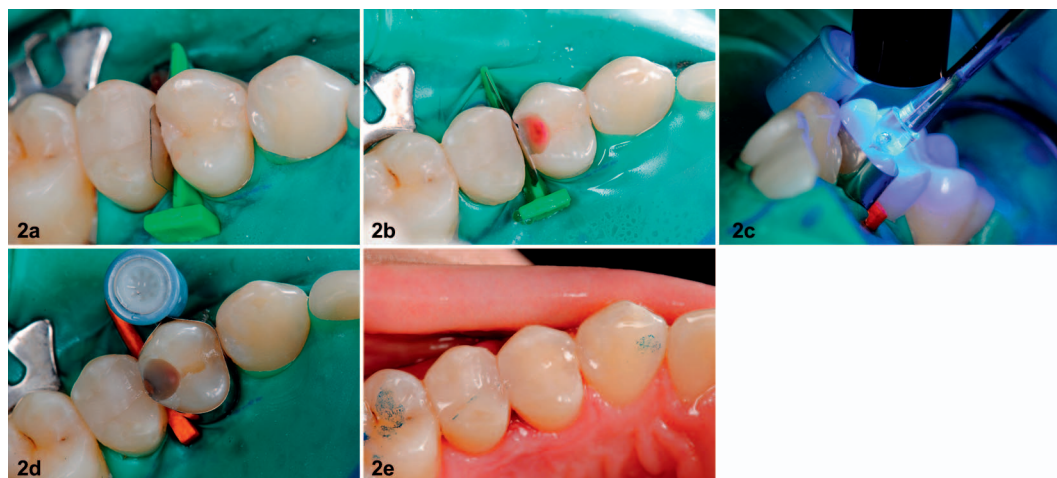


Figure 2. (a): Prewedging is done placing a thick wedge with a metal fin in the interproximal space to separate and protect the adjacent tooth. (b): The enamel without dentin support at the cavity floor is removed with a cylindrical bur that cuts only on the flat end. (c): The largest possible tip of LM-Contact Former (LMDental, Parainen, Finland) that can be introduced into the cavity is selected. The light tip should be placed on the end of the tip to minimize the loss of light intensity in the deepest areas of the cavity. (d): The composite resin is adapted to the cavity walls, unless in the area of the future proximal contact. This cavity is filled with a bulk-fill flowable resin-based composite until the last 2-mm occlusal layer that is restored with a micro-hybrid composite resin. (e): To polish the embrasures and round off the marginal ridge, discs with a square center are employed.

When the restoration is finished, the matrix band and the wooden wedge are removed, and then the restoration is polished. In the interproximal area, diamond-metal finishing strips (Diamond Finishing Strips cxds3-superfine, Edenta AG, Hauptstrasse, CH, Switzerland) are used because of their ability to pass more easily through the contact area without breaking. Also, they have a sawtooth central segment that is sometimes useful to introduce it from the occlusal side to polish the embrasures and round off the marginal ridge. Discs are also employed (Polishing Disc 12.7 DF-M, AXIS Dental, Crissier, Switzerland), placing the active face toward the head of the contra-angle and separating the teeth with a metal composite instrument (1/2 Trico composite instrument, Hu-Friedy Mfg Co) if there is not enough space for the disc. The discs used in this restoration have a square center, which prevents the mandrel from rotating without the disc rotating simultaneously. Finally, the polishing of the interproximal surface and the effectiveness of the proximal contact are checked with dental floss. If it frays, it will indicate irregularities that must be corrected. When the polishing is completed, the rubber dam is removed, and the occlusion is evaluated and adjusted as indicated. It is necessary to repolish the surfaces that required occlusal adjustment.

POTENTIAL PROBLEMS

There are no clinical studies that evaluate the efficacy of this technique.

In large Class II restorations, it is possible that the widest tip of the LM-Contact Former does not adjust to the proximal box. In these cases, it will be necessary to previously cure the composite resin in the medial wall to shorten the mesiodistal distance of the cavity.

The LM-Contact Former system has only four tip sizes. It would be necessary to have more dimensions to adapt to any cavity size.

SUMMARY AND ADVANTAGES AND DISADVANTAGES

This is a simple technique to restore Class II restorations with a composite resin and obtain tight proximal contacts using noncontoured circumferential matrix bands. Using the tips of the LM-Contact Former system, the composite resin is compacted onto the walls of the proximal box, and then the remaining cavity is filled with a bulk-fill flowable resin-based composite.

Advantages

- Proximal contacts obtained are tight, and the ideal anatomical shape of the proximal tooth surface is restored to recover its functionality.
- Appropriate contact between two adjacent teeth is essential to maintain tooth position and dentition stability, provide a food spillway, and facilitate hygienic cleaning. The relationship between contact type and food impaction has been confirmed,

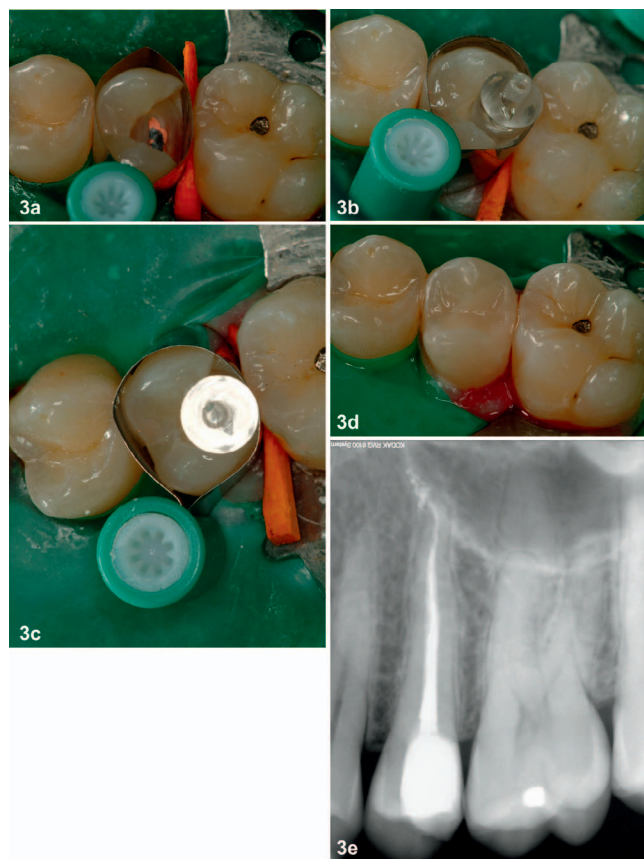


Figure 3. (a): A metallic noncontoured circumferential matrix band is adapted with the Hawe SuperMat system (Kerr, Orange, CA, USA) and a wooden wedge closely to the gingival contour of the cavity. (b): The corresponding tip of LM-Contact Former (LMDental, Parainen, Finland) to the cavity size is tried. (c): When inserting the tip with pressure, the matrix band is adapted to the adjacent tooth, and as a consequence, a tight proximal contact will be obtained. (d): Composite resin Class II restoration finished. (e): The proximal contact is located in the junction of the occlusal third with the middle third of the proximal surfaces.

reinforcing the significance of optimal proximal contact in preventing recurrent decay and periodontal problems.

- The placement and adjustment of a circumferential matrix band are simpler than that of a sectional matrix band, because it is easily deformed when placed, especially when it has not achieved a sufficient separation between teeth.⁹
- When inserting the tip into the cavity, the tip exerts pressure on the composite resin, which is pressed against the walls, avoiding the formation of voids.
- It is not necessary to place the composite resin in layers as in the incremental technique or the traditional centripetal technique.

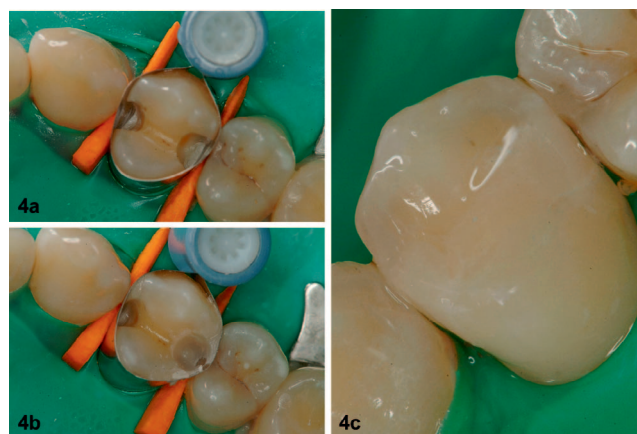


Figure 4. (a): Three-surface Class II cavity in 2.4. The matrix band must remain below the cavity floor, adapted closely to the gingival contour of the cavity. (b): The first of the proximal boxes is restored, followed by the other box. (c): Final aspect of the three-surface Class II restoration.

Disadvantages

- In teeth with large coronal destruction, this technique may not be indicated.
- If a lot of the composite is placed in the bottom of the cavity, when the tip is inserted, the excess goes up to the occlusal area. It can be removed before polymerizing or during the finishing and polishing of the restoration.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Department of Surgery and Medical and Surgical Specialties, Faculty of Medicine and Dentistry, University of Santiago de Compostela.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Clinical Research

Gingival Health of Porcelain Laminate Veneered Teeth: A Retrospective Assessment

R Arif • JB Dennison • D Garcia • P Yaman

Clinical Relevance

Porcelain laminate veneers offer a reliable and successful esthetic treatment, maintaining the long-term gingival health of the surrounding tissues.

SUMMARY

Statement of Problem: The long-term effect of the presence of porcelain laminate veneers (PLVs) on the health of the surrounding gingival issues is not available in the restorative literature.

Purpose: To assess the long-term effect of PLVs on the health of the surrounding gingival tissues. A secondary aim was to correlate gingival crevicular fluid (GCF) scores with clinical parameters used for gingival health assessment in teeth treated with PLVs.

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Methods and Materials: Patients who received PLVs placed at the Graduate Restorative Clinic within a seven- to 14-year period were recalled for clinical evaluations. Periodontal measurements including gingival index (GI), periodontal pocket depth (PPD), gingival recession (GR), and clinical attachment level (CAL) were measured using a standard probe and indices. Gingival Crevicular Fluid (GCF) was measured with a Periotron machine (Periotron 8000, Oraflow Inc), using Periopaper (Periopaper Gingival Fluid Collection Strip, Oraflow Inc.) for fluid collection. Photographs of any observed clinical defect were taken. Data were tabulated using Excel 2010 (Microsoft Corp). Statistical analysis for all descriptive statistics was performed using SPSS 21 (SPSS Software, IBM Corp.) and Stata SE 13 (Stata Software, StataCorp). Repeated-measures analysis of variance (ANOVA) was done to test for statistical significance of the mean pocket depths between the restored and unrestored surfaces of the veneered teeth. The significance level for all tests was $p < 0.05$. Pearson's correlation coefficient was performed for testing statistical significance between GCF and GI and between GCF and PPD.

Results: The frequency distribution of the GI included 47 PLVs (43%) with normal gingiva, 16

(15%) with mild inflammation, and 46 (42%) with moderate inflammation and bleeding on probing. The average PPD on the facial surface of the maxillary and mandibular PLVs was 2.17 mm and 2.16 mm, respectively. On the lingual surface, the average PPD was 2.10 mm for maxillary and 2.22 mm for mandibular PLVs. Gingival recession was seen in 27% of the evaluated PLVs. The repeated-measures ANOVA revealed $p \geq 0.136$, showing no statistical difference in the mean pocket depths between restored facial and unrestored lingual surfaces of the veneered teeth. A moderate correlation ($r=0.407$) was found between GCF and GI, which was significant at $p < 0.001$. No correlation ($r=0.124$) was found between GCF and PPD, which was not significant at $p=0.197$.

Conclusions: Gingival response to the evaluated PLVs was in the satisfactory range, with overall GI scores ranging between normal and moderate inflammation, pocket depths ranging from 1 to 2 mm, and recession present in 27% of the evaluated PLVs. No statistically significant difference was found between the mean pocket depths of the restored and unrestored surfaces of veneered teeth ($p \geq 0.136$). A moderate correlation was found between GCF and GI.

INTRODUCTION

The popularity of the bonded porcelain laminate veneer (PLV) has been consistently on the rise.¹ Over the span of 15 years, it became the second most requested porcelain restoration in the United States.¹ PLVs were introduced as a more conservative alternative to complete crowns. It is important for clinicians to understand the long-term clinical performance of these restorations. Gingival response to these restorations is of equal importance to the longevity of the restorations themselves. Since the advent of PLV treatment, a number of investigations with evaluation times ranging from 5 to 20 years have confirmed the favorable clinical performance of these restorations.²⁻⁶ In addition, with PLVs, the maintenance of esthetics has been reported to be excellent with high patient satisfaction.⁷

Porcelain is considered the most esthetic and biocompatible material in dentistry with the ability to imitate sound enamel.⁷ Based on these observations, one would expect no or even a positive reaction of the marginal gingival tissues toward porcelain veneers.⁷ Clinical performance is tied to gingival health; therefore, studies have quantified and ex-

amined gingival recession, pocket depth, and gingival index (GI) in relation to PLV treatment.^{4,8} Studies have investigated the effect of different restorations on gingival health with respect to margin placement, finish, and polish of the restorations.⁹⁻¹³ Researchers have evaluated the correlation between gingival crevicular fluid (GCF) and periodontal indices, with some reporting a correlation between Periotron readings and clinical parameters^{14,15} and some reporting no correlation.^{16,17}

Short- to medium-term studies ranging from four to seven years have reported satisfactory gingival response to PLVs.^{2,3,18,19} However, long-term clinical studies looking at the effect of the presence of PLVs on the health of the surrounding gingival tissues are not available. The present study aimed to bridge that gap and to provide, within its limitations, some information on this topic, which is still lacking in the literature. Therefore, the goal of this study was to evaluate long-term gingival health in response to PLVs, using clinical metrics for measuring gingival health. A secondary aim was to correlate GCF with clinical parameters used for gingival health assessment in teeth treated with PLVs. The null hypothesis tested was that the presence of PLVs will have no adverse effect on the gingival health seven to 14 years post cementation.

METHODS AND MATERIALS

The study was approved by the institutional review board and conducted according to approved guidelines. The inclusion criteria were subjects with PLVs placed within a seven- to 14-year period at the Graduate Restorative Clinic. These patients were identified by chart review and invited to participate. This was a sample of convenience, since all veneers were included and that number was fixed by previously performed clinical procedures over the stated time period. Patients were excluded if PLVs had fractured, been replaced by a new restoration, or if the veneered tooth was otherwise lost. In addition, patients could not be undergoing periodontal treatment at the time of veneer placement. Informed consent and updated medical history were obtained from those responding to the invitation. One calibrated evaluator recorded all measurements and photographs. A data collection form was used to record clinical parameters, which included the following.

The GI was obtained for three sites on each tooth—mesiofacial, facial, and distofacial—using the index scale described by Loe and Silness²⁰ and a periodontal probe (UNC Periodontal Probe, Hu-

Friedy Mfg Co, Chicago, IL, USA), recorded to the nearest millimeter. Periodontal pocket depth (PPD) was measured using the same periodontal probe on six sites per tooth: mesiofacial, facial, distofacial, distolingual, lingual, and mesiolingual. The main purpose of recording the lingual measurements was to compare them with the values of the facial surface and evaluate whether the presence of PLV on the facial surface made a difference in the PPD compared with the lingual surface. Since baseline information was not available, accurate gingival recession could not be recorded. Therefore, anatomic crown exposure was recorded on three sites per tooth, mesiofacial, facial, and distofacial, as the distance in millimeters from the facial margin of the veneer to the cemento-enamel junction as fixed reference points. Clinical attachment level (CAL) was determined for three sites on each tooth, mesiofacial, facial, and distofacial.

For GCF volume, the area of the tooth to be examined was isolated with cotton rolls and gently dried with an air syringe. An absorbent paper strip (Periopaper gingival fluid collection strips, Oraflow Inc, Hewlett, NY, USA) was placed 1 mm into the mesial gingival sulcus of the tooth for 10 seconds to dry the crevicular sulcus and then discarded. Immediately, another paper strip was placed for 30 seconds (standard time for pooling GCF) and positioned in the Periotron (Periotron 8000, Oraflow Inc, Hewlett, NY, USA) to obtain the numerical readout.²¹ A comprehensive calibration was done at the beginning of the study, then once per month and at any point during the study when an interim calibration was out of range. In addition, calibration was done according to manufacturer instructions immediately prior to taking readings on a participant. A log sheet was kept to record these readings and to validate sampling values. Once a reading was recorded for a PLV, the recorded score was divided by the mean of the calibration readings. The value obtained represented the GCF volume for each individual veneer. Photographs of any observed clinical defect were taken (Rebel XSI, Canon Inc, Melville, NY, USA).

Data were tabulated using Microsoft Office Excel 2010 (Microsoft Corp, Redmond, WA, USA). Statistical analysis for all descriptive statistics was performed using SPSS 21 (SPSS Software, IBM Corp, Armonk, NY, USA) and Stata SE 13 (Stata software, StataCorp LLC, College Station, TX, USA). Repeated-measures analysis of variance (ANOVA) was used to test for statistical significance of the mean pocket depths between the restored and

Table 1: *Distribution, Patient Demographics, and Location of Evaluated Porcelain Laminate Veneers*

Description	No.
Number of patients	24
Number of evaluated veneers	109
Number of male patients	7
Total number of restorations in male patients	24
Number of female patients	17
Total number of restorations in female patients	85
Location of veneers	
Number of mandibular	31
Number of maxillary	78
Distribution of veneers	
Central incisors	37
Lateral incisors	38
Canines	34

unrestored surfaces of the veneered teeth. This repeated-measures ANOVA took into account the subject effect. The significance level for all tests was $p<0.05$. Pearson's correlation coefficient test was run to test the statistical correlation between GCF and GI and between GCF and PPD.

RESULTS

Record review and patient contact resulted in 26 patients with 114 PLVs presenting for examination. Of these, two patients with five PLVs were excluded, four PLVs had fractured and been turned into complete crowns, and one was turned into a complete crown to serve as an abutment for a removable partial denture. These were not included in the evaluation sample. Therefore, the study evaluated 109 veneers in 24 patients. Results of patient demographics, number of participants, total number of veneers evaluated, and location of the PLVs are given in Table 1. The frequency distribution of GI scores according to arch, PPD for restored facial and unrestored lingual surfaces of the veneered teeth, gingival recession by tooth surface, and CAL per arch are presented in Table 2. The GI scores recorded for the mandibular arch were higher compared with the maxillary arch ($p<0.0001$). The average PPD on the facial of the maxillary PLVs was 2.17 mm and on mandibular PLVs was 2.16 mm. On the lingual surface, the average PPD was 2.10 mm for maxillary PLVs and 2.22 mm for mandibular PLVs. The repeated-measures ANOVA revealed no significant difference in the mean pocket depths between the restored and unrestored surfaces, either overall or in the interaction with the subject (Table

Table 2: Frequency Distribution of GI Scores per Arch, PPD for Facial and Lingual Surfaces of Evaluated Porcelain Laminate Veneers, GR by Tooth Surface, and Average CAL per Arch

Gingival Index (GI)						
Arch	Score ^a	Inflammation		Frequency (n)	Percentage	
Maxillary						
	0	Normal		43	55%	
	1	Mild		11	14%	
	2	Moderate		24	31%	
	3	Severe		0	0%	
	Total			78		
Mandibular						
	0	Normal		4	13%	
	1	Mild		5	16%	
	2	Moderate		22	71%	
	3	Severe		0	0%	
	Total			31		
Periodontal pocket depth (PPD)						
PPD (mm)	Facial			Lingual		
	MF	F	DF	ML	L	DL
1	3	25	4	0	14	3
2	70	76	63	79	91	78
3	36	8	41	30	4	28
4	0	0	1	0	0	0
Gingival Recession (GR)						
Surface		No Recession, n (%)			Recession, n (%)	
MF		93 (85%)			16 (15%)	
F		84 (77%)			25 (23%)	
DF		98 (90%)			11 (10%)	
Average Clinical Attachment Level (CAL)						
Surface		Maxillary Arch			Mandibular Arch	
MF		2.5			2.3	
F		2.07			2.06	
DF		2.53			2.25	

^a Score = Loe/Silness GI index.
MF=mesio-facial; F=facial; DF=disto-facial; ML=mesio-lingual; L=lingual; DL=distolingual

^a Score = Loe/Silness GI index.

MF=mesio-facial; F=facial; DF=disto-facial; ML=mesio-lingual; L=lingual; DL=distolingual

3). The teeth most likely to show recession in this study were maxillary lateral incisors (n=12) followed by canines (n=11) and central incisors (n=6). Figure 1 illustrates examples of veneered teeth exhibiting recession. With respect to the arch of the 29 PLVs exhibiting recession, 26 (24%) were maxillary PLVs and three (2.75%) were mandibular.

It was observed that the higher the GI score, the higher the value for GCF (Figure 2). Pearson's correlation coefficient revealed a coefficient (*r*) value

Table 3: Repeated-Measures Analysis of Variance for Mean Pocket Depths Between Restored and Unrestored Surfaces of the Veneered Teeth

Effect	Num DF	Den DF	F Value	Pr > F
Subject	24	84	7.37	<0.0001
Surface	1	84	1.03	0.314
Subject × Surface	24	84	1.39	0.136

Num DF, Number of Degrees of Freedom; Den DF, Denominator of Degrees of Freedom; F Value, F statistic; Pr, P value

of 0.407, signifying a moderate correlation between GCF and GI, which was significant at $p < 0.001$. For GCF and PPD, higher scores were observed for 2-mm, as opposed to 3-mm, pocket depths (Figure 3). A coefficient (*r*) value of 0.124 revealed that there was no correlation between GCF and PPD, which was not significant at $p = 0.197$.

DISCUSSION

To the authors' knowledge, this is the only study evaluating the long-term effect of PLVs on the health of the surrounding gingival tissues and the only one to extend the follow-up time to up to 14 years. The null hypothesis that the presence of PLVs will have no adverse effect on the gingival health after seven to 14 years postcementation was accepted. The study found that, overall, of the 109 evaluated PLVs, 47 (43%) had normal gingiva with no bleeding on probing (BOP), 16 (15%) had mild inflammation, and 46 (42%) had moderate inflammation with BOP. None of the evaluated veneers had overt gingivitis with spontaneous bleeding. It was observed that as the margins of the veneers got closer to the gingiva, there was an increased tendency for BOP, and this observation is analogous to results presented in earlier studies.^{4,8} Most of the veneers had a PPD of 1 to 2 mm, which indicated normal health of the surrounding tissues. The present study used the lingual gingiva of the restored veneered teeth as a comparison, which was previously described.⁸ In another study, when PPD was measured on all tooth surfaces, no systematic differences were found when comparing measurements of the buccal versus the lingual maxillary anterior tooth surfaces in non-restored teeth.²² The present study found no significant difference in the mean pocket depths between the restored facial and unrestored lingual surfaces of the veneered teeth. This finding has been reported previously.⁸ A similar observation was also reported by an evaluation looking at class V composite restorations and class V glass ionomers restorations.^{14,23} It is of interest to note that one study found periodontal indices, including pocket depth

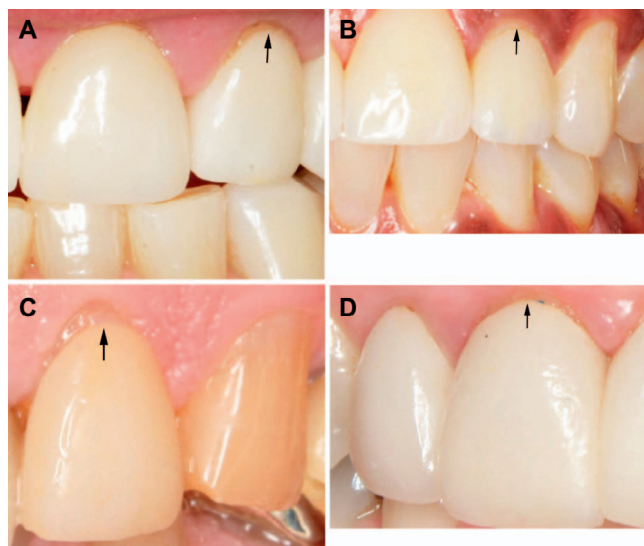


Figure 1A–D. Examples of facial recession observed during recall appointments.

and bleeding index, to be higher for porcelain fused to metal crowns compared with porcelain veneers.⁸

The present study found gingival facial recession in 27% (n=29) of the evaluated PLVs, and the reference for measurement was from the facial margin of the PLV to the free gingival margin. The same reference was used by another study, which reported 31% recession after 10 years.⁴ Previously, a study reported an increased tendency for gingival recession of the veneered teeth after 5 years, which became more obvious at the 10-year recall.^{5,24} The incidence of recession was more common in the maxillary PLVs; however, this may be due to skewed sample size. Maxillary lateral incisors followed by canines were found to be the teeth most likely to exhibit recession. The observed recession is most likely a result of time, which has been previously reported.⁴ It has been found that recession may be seen on both veneered and nonrestored teeth.^{4,5} In a longitudinal analysis, it was reported that even in subjects with a high standard of oral hygiene, buccal recession occurred frequently and the number of subjects presenting with recession increased with age.²⁵ It is of interest to note that patients and dental professionals have been reported to have different perceptions about esthetics related to gingival margin position, with patients not being able to observe recession up to 2 mm and dental professionals tending to rank lower esthetic satisfaction with the presence of recession.²⁶ In addition, operator variability and overzealous cord packing could have caused the margins to recede. It has also been suggested that occurrence of recession is

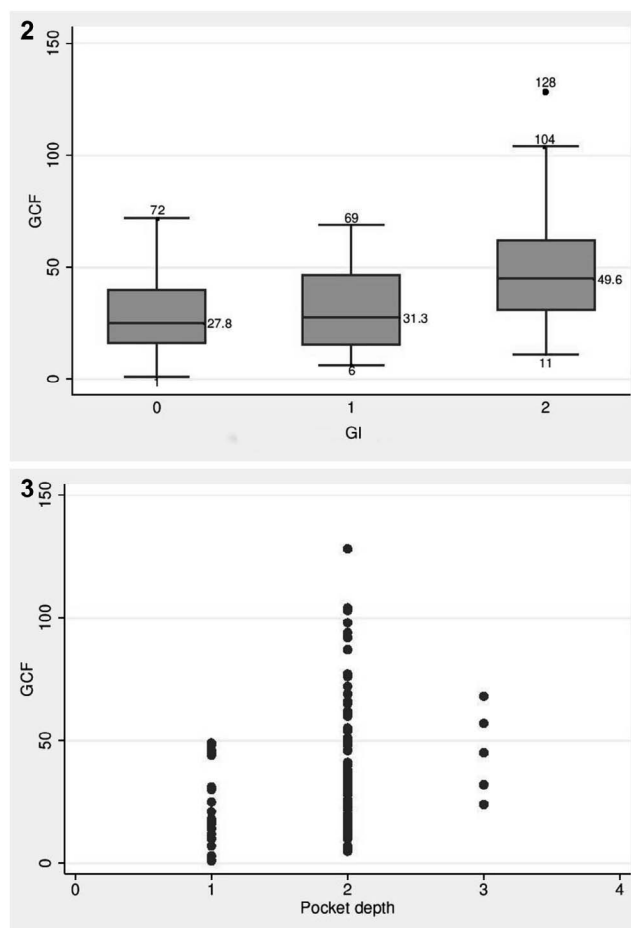


Figure 2. Box plot displaying data values for gingival index, gingival crevicular fluid. For each gingival index score (0, 1, 2) minimum, maximum, mean scores of gingival crevicular fluid (GCF) are displayed with I- bars.

GCF= gingival crevicular fluid; GI= Loe and Silness gingival index

Figure 3. Scatter Plot displaying scores of gingival crevicular fluid, periodontal pocket depth (mm).

GCF= gingival crevicular fluid; Pocket depth= mm

significantly related to equigingival or subgingival margins.⁴ Therefore, wherever possible, margins of the veneers should be kept supragingival without violation of the biological width in order to expect the best gingival response.

The CALs are used not only to indicate past destruction of the periodontal attachment apparatus but also to monitor the progression of periodontitis. However, accurately assessing CAL can be difficult in the presence of inflammation and recession. Even though no overgrowth of tissue due to inflammation was observed, there was a trend for recession, and therefore, an accurate measurement of the CAL was difficult.

A second aim of this study was to correlate GCF with clinical parameters used for gingival health assessment in teeth treated with PLVs. The study found a moderate correlation between GCF and GI ($r=0.407$), which was significant at $p<0.001$. This has also been observed by another study.¹⁵ On the contrary, some studies found no correlation between GCF and GI.^{16,17} It was observed in this study that the GCF scores of some PLVs were high, but the GI scores of the same PLVs were indicative of normal gingival health. This finding has also been observed by another study that suggested clinical indices to be crude for assessment of inflammation or the technique for sampling of GCF volume to be imprecise.¹⁶ Lower GCF scores could have been obtained in this study, had the PLVs of patients been evaluated after their regular hygiene visit or if the supragingival plaque had been removed prior to evaluation. The presence of supragingival plaque has been reported to significantly elevate GCF scores.²⁷ The patients were instructed to get regular prophylaxis along with reinforcing the importance of brushing and flossing. Another reason for the higher GCF values seen in this study could be that a second sample of GCF was not collected. It has been suggested that the first sample is more closely related to the dimension of the crevicular space and the second one is more likely to be associated with the degree of inflammation.¹⁶ In addition, the volume of GCF sample could be too small for accurate readouts, as it has been suggested that for lower volumes, errors in measurement may be high due to problems with evaporation, strip placement, and measurement technique, rather than errors related directly to the Periotron itself.²⁸

This study found no correlation between GCF and PPD scores ($r=0.124$). However, one study did find a correlation between them.¹⁵

This study is not without limitations. These include the retrospective nature of the study, the sample being one of convenience, and the limited information available on certain aspects of the PLVs, including but not limited to the specific material used for fabrication of the veneers and operator variability. However, within the limitations of this study, it was found that after seven to 14 years of clinical service, the health of gingival tissues surrounding PLVs is not adversely affected by their presence.

CONCLUSIONS

Within the limitations of this long-term retrospective evaluation of gingival health around PLVs, the

gingival response to the evaluated PLVs was in the satisfactory range, with overall GI scores ranging between normal to moderate inflammation, PPD scores ranging from 1 to 2 mm, and mild recession present in 27% of the evaluated PLVs. The teeth most likely to show recession in this study were maxillary lateral incisors. No difference was found in the pocket depths between the restored facial and unrestored lingual surfaces of the veneered teeth $p\geq 0.136$. A moderate correlation was found between GCF and GI ($r=0.407$), which was significant at $p<0.001$. No correlation was found between GCF and PPD ($r=0.124$), which was not significant at $p=0.197$.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Michigan. The approval code for this study is HUM00066344).

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Clinical Study of Bleaching Gel Storage Temperature on Tooth Color and Sensitivity

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Clinical Relevance

Bleaching gels stored at room temperature or kept refrigerated before immediate use showed similar results for color stability, tooth sensitivity, and pH. However, the consistency of the gels when dispensed may be affected by storage temperature.

SUMMARY

Objective: The objective of this triple-blind, split-mouth, randomized clinical trial was to evaluate the bleaching efficacy and tooth sensitivity of an in-office bleaching agent submit-

ted to different storage temperatures (room temperature at $21.04^{\circ}\text{C} \pm 3.13^{\circ}\text{C}$ or refrigeration at 5°C).

Methods and Materials: Thirty volunteers were selected who had central incisors with color A2 or higher. The volunteers' maxillary hemi-arches received either the bleaching treatment with room temperature or refrigerated storage temperatures (two sessions of 3×15 minutes, one-week interval). Color variation was evaluated by subjective (Vita Classic and Vita Bleachedguide) and objective methods (Vita Easyshade spectrophotometer). Tooth sensitivity was evaluated with the visual analog scale (0–10) and the numerical rating scale (five points). The consistency of bleaching gels was evaluated by flow test, and pH was measured, both in triplicate. Color variation (SGU) and ΔE were analyzed by paired *t*-test ($\alpha=0.05$). The absolute risk of pain was assessed by McNemar test ($\alpha=0.05$), data from the numerical rating scale by the Wilcoxon signed-rank test ($\alpha=0.05$), and visual analog scale by paired *t*-test. Comparison between the times within each group was analyzed by Friedman test. Gel consistency and pH were analyzed by one-way analysis of variance and Tukey post-test.

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Results: Regarding the absolute risk of tooth sensitivity, no significant difference was observed between the groups. The relative risk for tooth sensitivity was 1.13 (95% confidence interval 0.70–1.82). Both tooth sensitivity scales were statistically similar. The results of the subjective evaluation (Vita Classic: $p=0.73$, Vita Bleachedguide: $p=1.00$) and the objective evaluation ($p=1.00$) of bleaching efficacy corresponded to the hypothesis of equality between groups after bleaching. Both pH values were around 7, and for the consistency test, there were significant differences between the groups ($p=0.002$).

Conclusions: Storage temperature of the analyzed in-office bleaching agent had no influence on tooth color effectiveness and tooth sensitivity.

INTRODUCTION

The quest for lighter teeth has become increasingly frequent in dental practice.¹ Since Haywood and Heymann² introduced whitening in 1989, the development of conservative and effective treatments, with rapid results, has made dental whitening one of the most popular procedures in modern dentistry.^{3,4}

Dental dyschromia may be caused by intrinsic and/or extrinsic factors.⁵ The bleaching technique used for each type of dyschromia may vary. However, all techniques are based on the use of hydrogen or carbamide peroxide, with concentration and application time changing according to the technique used: home or in-office therapy. With the in-office technique, activation by heat or light may be used to accelerate and enhance bleaching, as widely discussed in the literature.⁶⁻⁸ However, some recent studies have suggested that the use of light as a bleaching agent activator could be detrimental, as it may possibly cause an increase in the temperature of the pulp chamber⁹ and the risk of tooth sensitivity.¹⁰

Bleaching agents are described in the literature as chemically unstable substances,¹¹ their stability being related to local factors such as temperature, light, moisture, pH, and impurities.¹² Increasing the temperature helps degrade hydrogen peroxide by accelerating the release of the hydroxyl radicals from the peroxide, according to the following equation: $\text{H}_2\text{O}_2 + 211 \text{ kJ/mol} \rightarrow 2 \text{HO}\cdot$. For each additional 10°C increase in temperature there is an increase in the degradation rate of the peroxide in the order of 2.2. This increased release of hydroxyl radicals (called thermocatalysis) may increase bleaching

efficacy.¹³ However, light activation does not make any difference in tooth lightening, although the heat from the external light units considerably increases the temperature of the hydrogen peroxide.^{6,7,10,13}

Other authors found that increasing temperature had a negative effect on the pH stability of bleaching agents.¹⁴ Bleaching gel stored in a refrigerator ($4^\circ\text{C} \pm 1^\circ\text{C}$) showed higher pH than bleaching gel stored at room temperature ($23^\circ\text{C} \pm 1^\circ\text{C}$). The value of pH may decrease when there is an increase in the ions of H^+ , as maintaining the product at higher temperatures may induce dissociation of some components, leading to a high concentration of H^+ ions and consequent reduction of pH in the long term. Bleaching agents with acid pH have a higher rate of oxygen ions (O^{2-}), while agents with more neutral pH have a higher rate of perhydroxyl ions (HO_2^-).¹⁴ Furthermore, Marson and others concluded that the longer the bleaching gel is stored at higher temperatures (37°C), the higher the decrease in peroxide concentration compared with that of bleaching agents maintained between 5°C and 25°C after 6 months.¹⁵ These findings can be attributed to Le Châtelier's principle, which states that the effect of chemical equilibrium is related to some conditions, such as temperature; in other words, if there is a change in temperature, the position of the chemical equilibrium will change in one direction, where it will reduce stress, and thus restore a new chemical balance.¹⁶ One way to maintain the pH stability of the bleaching gels is to keep it refrigerated.^{14,15} However, some manufacturers report that there is no need to keep bleaching gel cool because ambient temperatures should be sufficient to ensure satisfactory chemical properties in hydrogen or carbamide peroxide gels. Others advise that the bleaching agents should be kept at ambient temperatures between 5°C and 25°C : the first value represents refrigeration, and the second is the average ambient temperature in several places. These factors play a key role during the shelf life of bleaching gels^{17,18} and during their transport and storage at dental offices.

The physical properties of the bleaching agent should also be discussed. These products should have good flowability to facilitate insertion, an adequate degree of elasticity, and high viscosity at low tension so the product remains in contact with the dental surface,¹⁹ thereby improving its effectiveness by increasing product contact with the tooth. Furthermore, it would prevent the substance from reaching other locations, since it is known that the bleaching gel may have adverse effects on adjacent oral

tissues.²⁰⁻²⁵ Also, some at-home and in-office bleaching agents use Carbopol as a thickener; in this type of component, temperature influences the rheologic characteristics,²⁶ which in turn, may change the bleaching gel viscosity.

The literature contains only *in vitro* data on the effect of temperature on the properties of bleaching agents. Storage temperature may influence the pH of these agents, as refrigerated gels had a higher pH than those kept at room temperature.¹⁴ Moreover, lower pH values may lead to more adverse effects on dental structures^{27,28} and enhanced tooth sensitivity.²⁹ However, there are no *in vivo* studies on the influence of storage temperatures on clinical parameters. Therefore, the objective of this triple-blind, split-mouth, randomized clinical trial was to evaluate the effect of an in-office bleaching agent stored at room temperature or refrigerated on bleaching efficacy and tooth sensitivity. The null hypotheses were that the storage temperature would not influence bleaching efficacy or tooth sensitivity.

METHODS AND MATERIALS

Study Design

This study was designed according to the CONSORT statement.³⁰ The study was conducted at the clinic of the School of Dentistry. All participants were informed about the nature and objectives of the study. Written informed consent was obtained from all participants before starting the treatment. This was a triple-blind and split-mouth randomized clinical trial with two experimental groups.

Participant Recruitment

Recruitment was performed by posting written advertisements on the university walls. All volunteer participants signed an informed consent form before being enrolled in the study.

Inclusion and Exclusion Criteria

Thirty nonsmoker patients between 18 and 40 years old were included, all with good general and dental health, healthy maxillary and mandibular anterior teeth, a good level of oral hygiene, no cervical lesions, no white spot lesions or caries, and no periodontal disease. The maxillary central incisors of each patient were evaluated, specifically the medium third of the facial surface, according to American Dental Association recommendations.³¹ These teeth presented color A2 or higher, according to the Vita scale (VITA Classic, VITA Zahnfabrik, Bad Säckingen, Germany) and Vita Easyshade (Easyshade;

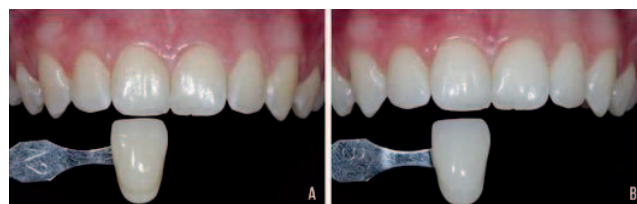


Figure 1. Initial color registration of the maxillary central incisors and their color 1 month after the procedure. (A): Baseline. (B): After one month.

Vident, Brea, CA, USA). All evaluations were conducted under the same illumination in the same room by the same two examiners, who recorded the initial color of the maxillary central incisors and their color 1 month after the procedure (Figure 1). For calibration purposes, five participants who were not included in the study sample participated in the training phase. The two examiners (DH and BFB), who were blinded to the allocation assignment, scheduled these patients for bleaching and evaluated their teeth against the shade guide at the baseline (one week after bleaching) and 30 days after the procedure. The two evaluators presented superior color-matching competency according to the ISO/TR 28642.³² This means they had an agreement of least 85% (Kappa statistic) before beginning the study evaluation (85% of correctly matched pairs of tabs in shade guides). If disagreements occurred during the evaluation, the evaluators needed to reach a consensus before the participant was dismissed.

The exclusion criteria were participants needing endodontic treatment or those with nonvital teeth, pregnant or breast-feeding women, participants with known allergies to bleaching materials or any other material used in this study, those taking anti-inflammatory, analgesic or psychotropic drugs, the presence of restorations in the anterior teeth, parafunctional habits, those with severe internal tooth discoloration (tetracycline stains, fluorosis, pulpless teeth), those who smoked, and those who had undergone tooth-whitening procedures.

During the first appointment, tooth sensitivity was measured using an air stream in the cervical region of the anterior teeth and vertical/horizontal percussion. Patients who demonstrated pain (on a verbal scale from 0 to 4) were excluded from the study.

Randomization and Group Allocation

Randomization was performed using opaque envelopes containing the group allocation, which was

revealed when opening the envelopes at the time of the bleaching procedure.

Blinding

This study was a triple-blind clinical trial, as the patients, the operator and the data analyst were kept blind.

Intervention

All patients received oral hygiene instructions and prophylaxis before initiating the bleaching procedure. The patients were randomly bleached in a split-mouth design according to the two storage temperatures ($n=30$): room temperature ($21.04^{\circ}\text{C} \pm 3.13^{\circ}\text{C}$) and refrigerated (5°C). Bleaching gels were allocated at their respective temperatures for one month before the study and were maintained until the end of the study, for 3 months. The product was used before the expiration date. Each patient served as his or her own control. After placement of a lip retractor (Arcflex, FGM, Joinville, SC, Brazil) the operator isolated the gingival tissue of the teeth to be bleached using a light-cured resin dam (Top Dam, FGM), which was light cured for 20 seconds on each three-tooth group (Radii-cal, SDI, Bayswater, Victoria, Australia). Another operator manipulated the bleaching gel, to ensure operator blinding. The researcher applied the 35% hydrogen peroxide gel (Whiteness HP Maxx, FGM), inserting the gel in a Luer disposable syringe (three 15-minute applications for both groups), in accordance with the manufacturer's directions. The researcher refreshed the in-office bleaching agent every 15 minutes during the 45-minute application period. Two bleaching sessions were performed one week apart.

Tooth Sensitivity Evaluation

Tooth sensitivity was evaluated during bleaching and up to one hour, 24 hours, and 48 hours after bleaching in both sessions. Participants were instructed to record the tooth sensitivity they experienced for each period listed earlier, using a five-point verbal numerical rating scale and a visual analog scale. For the numerical rating scale, participants had to choose one score from 0 to 4 to represent the intensity of tooth sensitivity using the following scale: 0 indicating no pain; 1, mild pain; 2, moderate pain; 3, considerable pain; and 4, severe pain.³³⁻³⁶ For the visual analog scale, participants had to mark a line perpendicular to a 10-mm line, where: 0 indicated no pain and the 10-mm end indicated severe pain.^{29,33,34,37}



Figure 2. Tip of the spectrophotometer placed in the labial perforation of the silicone matrix, which was used for individual evaluation of the right and left maxillary central incisors.

Sample Size

Sample-size calculation was based on the primary outcome of the study, color alteration (ΔE), measured using a spectrophotometer. With a significance level of 5% and power of 90%, 30 patients were required to detect a difference of means of three units of ΔE and standard deviation of two.³⁸⁻⁴⁰ The selected equivalence limit of $\Delta E=3.0$ is that at which color differences become clinically perceptible.

Color Evaluations

Initial and final color evaluations were performed at baseline (after bleaching) and one month later, respectively. Before baseline color evaluation, a preliminary impression of the maxillary teeth was taken using silicone impression material (Speedex; Vigodent S/A Ind. Com, Rio de Janeiro, RJ, Brazil). This impression served as a guide to standardize the color measurements using a VITA Easyshade spectrophotometer (Easyshade, Vident). A hole the size of the active tip of the Easyshade device was made in the facial surface of the silicon guide. The selected position corresponded to the middle third of the right and left central incisor facial surface (Figure 2). Color was determined using CIE $L^*a^*b^*$ parameters, where L^* indicates luminosity, varying from 0 (black) to 100 (white), and a^* b^* represent the axis-chromatic, where a^* is the measure along the red-green axis and b^* the measure along the yellow-blue axis.

The CIE $L^*a^*b^*$ values were measured in each patient. The differences between the baseline and each recall appointment (ΔE) were calculated according to the following equation: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.⁴¹

For the subjective evaluation, we used the shade guides VITA Classic and VITA Bleachedguide (VITA Zahnfabrik), where the VITA Classic was arranged from whitest to darkest levels. Although the VITA Classic is not a linear scale, it was arranged from the largest value (B1) to the smallest value (C4),^{10,42} representing a score for the purpose of analysis. The VITA Bleachedguide is a shade guide for dental bleaching, where it is already organized from the highest (0M1) to the lowest value (5M3).^{43,44}

Consistency Test

The consistency of the bleaching gels was evaluated by a flow test using two glass plates with constant load for a set time, as described by Panzeri and others.⁴⁵ Initially, 1 g of the bleaching gel was weighed and placed over a glass plate. Another glass plate was placed over the gel with a constant load of 761.75 g. After 5 minutes, the highest and lowest halo diameters resulting from the load were calculated using a digital caliper (Diginess, São Paulo, SP, Brazil). Measurements were performed in triplicate.

pH Measurement

The pH values were verified using a calibrated digital potentiometer (HI 221 Microprocessor pH Meter, Hanna Instruments, São Paulo, SP, Brazil), with a proton selective glass electrode.⁴⁶ To calibrate the pH meter, the standard operating procedure was followed: the electrode was inserted and agitated in a beaker with buffer solution (pH 6.86); the sensitivity of the apparatus was adjusted to 95% to measure 6.86. The electrode was removed, washed with distilled water, dried, and inserted into another beaker with 4.00 pH buffer solution. The calibration procedure was repeated, this time to assess the value of 4.00; after this, the calibrated pH meter was ready for use. The analysis of pH was performed in triplicate for each of the three samples of whitening gel used in this research. In a beaker, 1 g of bleaching gel was dissolved in 10 mL of distilled water.⁴⁵ Dispersion was achieved using a magnetic stirrer (Fisaton, São Paulo, SP, Brazil) for 5 minutes. Then, the proton selective glass electrode was introduced into the beaker containing the dissolved bleaching gel, avoiding touching the bottom of the bottle.

Statistical Analyses

The analyses followed the intention-to-treat protocol.³⁰ The statistician was blinded to the experimental groups. The absolute risk of tooth sensitivity was compared using the McNemar test ($\alpha=0.05$). The

relative risk and confidence interval for the effect size were also calculated.

Differences in the intensity of tooth sensitivity between the groups were analyzed using the Wilcoxon signed-rank test. Comparisons between each group were done by the Friedman test. Comparison of the intensity of sensitivity according to the visual analog scale from the groups at the two recall points was performed by paired *t*-test. For the comparisons between the recall times, the Friedman test was used. Color variation (Δ SGU and Δ E between baseline versus after one month) was analyzed by paired *t*-test. Gel consistency and pH were analyzed by one-way analysis of variance and Tukey post-test.

All analyses adopted a 5% level of significance (SigmaPlot 11.0, Systat Software, San Jose, CA, USA).

RESULTS

Characteristics of the Participants

A total of 30 patients were selected for this study (Figure 3), 33% of which were male. Age ranged from 18 to 40 years (mean age: 27.4 ± 6.9 years). The baseline color was 8.86 ± 2.78 SGU for the refrigerated group, 8.93 ± 2.79 SGU for the room temperature group (VITA Classic), 9.03 ± 1.44 SGU for the refrigerated group, and 8.96 ± 1.51 for the room temperature (VITA Bleachedguide).

Adherence to Protocol

All patients completed the bleaching treatment and returned to the recall appointments one month after bleaching.

Tooth Sensitivity

In terms of the absolute risk of sensitivity, no differences were observed between the groups, as shown in Table 1 ($p=0.29$). The relative risk was 1.13 (95% confidence interval, 0.70–1.82). Data on tooth sensitivity from both scales were statistically similar ($p>0.05$, Tables 2 and 3).

Color Evaluation

At the end of the bleaching procedure, variation of six SGUs was found for both groups using the VITA Classic guide and eight SGUs for the VITA Bleachedguide. The Δ E varied by approximately 12 units (Table 4), demonstrating significant color alteration. Comparisons between the two groups using the subjective evaluations (Vita Classic: $p=0.73$; Vita Bleachedguide: $p=1.00$) and the objec-

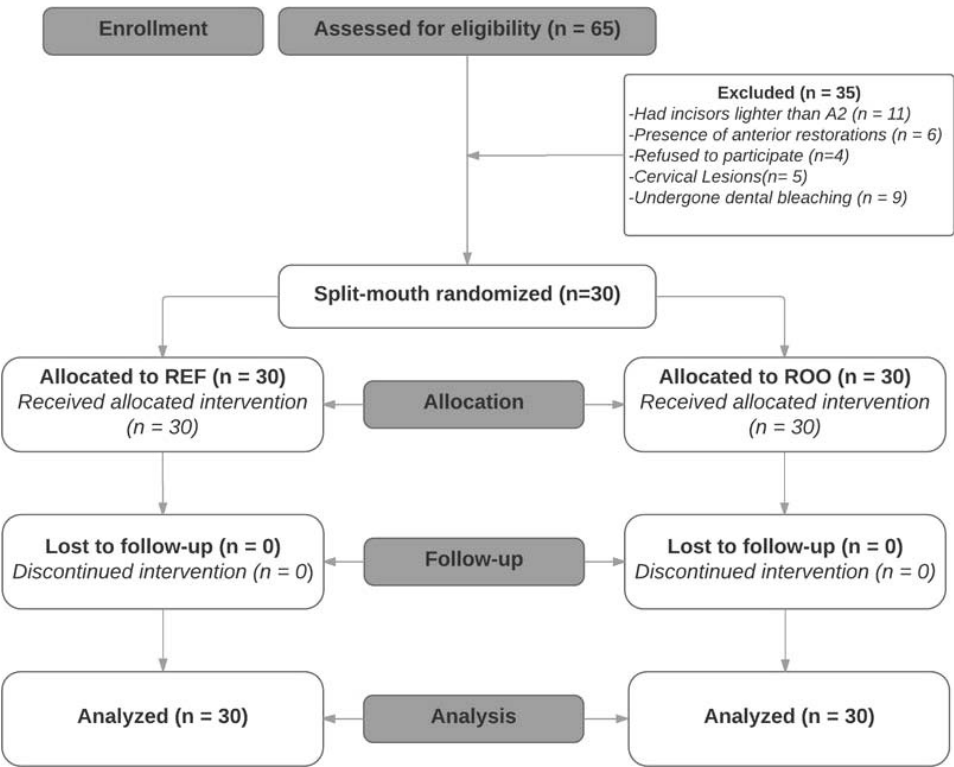


Figure 3. Flow diagram of study design phases, including enrollment and allocation criteria.

tive evaluation ($p=1.00$) showed no significant differences. There were no significant differences in tooth color between groups, regardless of the method used to evaluate tooth color (Table 4).

Consistency Test and pH

No significant differences in pH were observed between the groups according to Table 5 ($p=0.25$). All pH values were close to 7. The consistency test revealed significant differences between the groups ($p=0.002$), with the refrigerated group showing higher values than the room temperature group.

DISCUSSION

The present study confirmed the null hypothesis that the storage temperature of the bleaching gel did not influence the effectiveness of bleaching in terms

of tooth color. Both groups showed similar results after two 3×15 min sessions. Moreover, the subjective and objective evaluations demonstrated that both groups showed effective bleaching (Figure 4). The color change observed for VITA Classic was six SGUs, which is consistent with previous studies.^{29,34,42,47-49}

The null hypothesis regarding tooth sensitivity was confirmed. No differences were found between the two groups. Slightly lower sensitivity was observed for the group treated with refrigerated bleaching agent (0.3 ± 1.0) compared with the room temperature agent (0.7 ± 1.5). However, this result did not appear to be clinically relevant. The absolute risk of tooth sensitivity in this study was low and similar. On the other hand, other studies have presented higher absolute risks of sensitivity, varying from 67% to 100%.^{47,50-53} These differences may

Table 1: Comparison of the Number of Patients Who Experienced Tooth Sensitivity During the Bleaching Procedure in Both Experimental Groups, Absolute Risk and Relative Risk ^a				
Treatment	Tooth sensitivity (No. of patients)		Absolute risk (95% Confidence Interval)	Relative risk (95% Confidence Interval)
	Yes	No		
Room temperature	17	13	57 (39–73)	1.13 (0.70–1.82)
Refrigerated	15	15	50 (33–67)	

^a McNemar test ($p=0.29$).

Table 2: Medians and Interquartiles from Tooth Sensitivity Intensity in Different Recall Times Using the Numerical Rating Scale ^a

Recall Times	Refrigerated**	Room Temperature**	p Value*
During bleaching	0 (0 to 0) ^A	0 (0 to 2) ^A	0.64
1 hour after	0 (0 to 1) ^A	0 (0 to 1) ^A	1.00
24 hours after	0 (0 to 0) ^A	0 (0 to 0.75) ^A	0.09
48 hours after	0 (0 to 0) ^A	0 (0 to 0) ^A	0.38

^a Wilcoxon* and Friedman** tests; $p > 0.05$. Within each column, significant differences are represented by distinct uppercase letters.

be attributed to the formulations of the bleaching gels and other environmental factors. The presence of tooth sensitivity associated with bleaching procedures using hydrogen peroxide is well documented but is not completely understood. Alternatives are being investigated to avoid this adverse effect.^{29,33,35,37,38,48,53}

Hydrogen peroxide gels are described in the literature as chemically unstable compounds¹¹ and their stability has been related to physical factors such as temperature.¹² When temperature increases, it has a negative effect on pH stability.¹⁴ For this reason, it would be expected that the bleaching gel stored at refrigerated temperature showed lower tooth sensitivity, due to higher pH values. More acidic bleaching agents can lead to adverse effects on tooth enamel, such as a significant increase in surface roughness, wear of the dental enamel, and greater dental sensitivity.²⁷⁻²⁹ However, the bleaching gel of this study did not demonstrate these findings as pH did not vary by storage temperature. No variance of the bleaching gel pH according to the storage temperature may be due to a number of factors, including stabilizers, such as phosphoric or other inorganic acids, which allow prolonged storage that avoids short-term chemical rebalancing.⁵⁴

Loguercio and others²⁹ evaluated two bleaching gels with different pH values and concluded that

Table 3: Means and Standard Deviations of Tooth Sensitivity Intensity in Different Recall Times Using the Visual Analog Scale

Recall Times	Refrigerated	Room Temperature	p Value ^a
During bleaching	1.3±2.6 ^A	1.5±2.4 ^A	0.63
1 hour after	1.4±2.4 ^A	1.5±2.6 ^A	0.83
24 hours after	0.3±1.0 ^B	0.7±1.5 ^{A,B}	0.02
48 hours after	0.1±0.5 ^B	0.2±0.6 ^B	0.25

^a According to t-test. Within each column, significant differences are represented by distinct uppercase letters.

Table 4: Means and Standard Deviations of Δ SGU Obtained from Vita Classic and Vita Bleachedguide; Δ L, Δ a, Δ b, and Δ E Obtained by Easysshade; Baseline Versus One-month Measures, and p Values

Color Evaluation	Groups		p Value ^a
	Refrigerated	Room Temperature	
Subjective Evaluation (ΔSGU)			
Vita Classical	6.7±2.7	6.4±2.6	0.73
Vita Bleached	8.0±2.9	8.0±3.1	1.00
Objective evaluation (spectrophotometer–CIELab parameter)			
ΔL	5.9±5.5	5.5±5.8	0.77
Δa	-2.3±1.9	-2.2±1.9	0.72
Δb	-8.9±3.9	-9.0±3.7	0.91
ΔE	12.0±3.7	12.2±4.1	1.00

^a Paired t-test

^a Paired t-test.

neutral pH gels help to reduce tooth sensitivity. These findings may explain why there was no significant difference between the experimental groups in this study, as pH analysis of the gels demonstrated that both gels had an almost neutral pH, and thus the risk of tooth sensitivity was similar.

One of the factors that may have influenced the lower tooth sensitivity values was that bleaching treatment with 35% hydrogen peroxide was done only on the maxillary teeth. Other studies used 35% hydrogen peroxide on both the maxillary and mandibular arches.^{33,50-53} The mandibular teeth are more likely to have a high risk of sensitivity, as reported by Haywood,⁵⁵ because the teeth are smaller. Because they have a thinner layer of enamel and dentin, the hydrogen peroxide can penetrate the dental pulp more rapidly, thus reducing the time required for a pulpal response compared with that in a tooth with thicker enamel and dentin.⁵⁶ Histopathological studies conducted by Costa and others revealed notable damage in pulp tissue of the mandibular incisors after whitening compared with premolars, which are bulkier.⁵⁶ In the present study, bleaching treatment of the mandibular arch was

Table 5: Means and Standard Deviations of Flow Test (in Millimeters) and pH Measurements Obtained in Triplicate^a

Group	Flow (mm)	pH
Room temperature	98.81±2.20 ^a	6.88±0.29 ^c
Refrigerated	83.66±3.28 ^b	6.62±0.16 ^c

^a Lowercase letters showed significant differences between lines ($p < 0.05$). One-way analysis of variance and Tukey post-test.

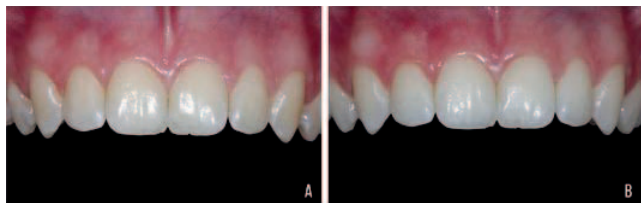


Figure 4. Color change observed in a patient at different evaluation periods. (A): Baseline. (B): After one month.

available to all patients, and the tooth sensitivity data were not collected. In addition, all patients chose to use an individual tray and at-home bleaching for the mandibular arch after the end of this clinical trial.

Another factor that may have influenced some studies that reported greater tooth sensitivity is the use of 35% hydrogen peroxide for an uninterrupted 45-minute period, contrary to the manufacturer's instructions. An example is a study in which the risk of tooth sensitivity was 90% (compared with 57% in the current study in which the in-office bleaching agent was refreshed every 15 minutes during the 45-minute application period).⁵¹ The type of whitening technique may also determine tooth sensitivity; Basting and others found the highest risk of tooth sensitivity in the group using at-home 20% carbamide peroxide (71.4%) and a lower risk of sensitivity in the groups who used 35% and 38% hydrogen peroxide in office (47% and 15%, respectively).⁵³ The in-office bleaching agent contained desensitizing agents, a fact that may have supported the lower tooth sensitivity results.

In the flow test, the refrigerated bleaching gel showed higher consistency than the room temperature gel. The temperature rise relaxes the polymer network of the Carbopol thickener, decreasing gel viscosity. The temperature decrease could restore the prerelaxed network, increasing the bleaching agent viscosity.²⁶ This may be seen as an advantage, as better control would be achieved when the gel is applied. The higher viscosity ensures that the bleaching gel maintains contact only with the facial surface of the teeth, preventing the substance from reaching other sites; this is advantageous as it is known that bleaching gel may have adverse effects on adjacent oral tissues.²⁰⁻²⁵

As already mentioned, rheologic features improve bleaching effectiveness by increasing the product contact with teeth.¹⁹ The viscosity of the whitening agent will affect product distribution and how it flows from the syringe. The viscosity test demon-

strated that the gel subjected to refrigerated temperature possessed a higher viscosity; thus, better application control was ensured. Otherwise, the gel at room temperature, in which viscosity was lower, should require more caution when applying, in order to guarantee that it stays in place on the tooth surface and does not reach adjacent tissues. However, the higher viscosity of the refrigerated bleaching gel may hinder its application once it becomes more resistant to flow from the syringe and may not distribute the product equally on the tooth surface. This may be one of the main reasons why manufacturers recommend leaving the product at room temperature before application.

It is worth mentioning that further clinical studies are required to evaluate other bleaching gels with different pH values and at higher room temperatures, as some locations have average temperatures above 25°C.

CONCLUSION

The results of this study show that the storage temperature conditions studied had no influence on color stability or tooth sensitivity for the analyzed in-office bleaching gel. The pH values were similar for both groups; however, the refrigerated bleaching gel was more viscous than the gel kept at room temperature, which may suggest an improvement during application of the product. Furthermore, hydrogen peroxide gel should not be stored at room temperature in places where room temperature is higher than 25°C. In these conditions, the in-office bleaching gel must be kept refrigerated to avoid deleterious effects.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ethics Committee of the Centro de Ensino Superior dos Campos Gerais. The approval code for this study is: 1.437.224. This study was registered in the Brazilian Clinical Trials Registry under the identification number RBR-5td5hg.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Whitening Efficacy of Chewing Gum Containing Sodium Metaphosphate on Coffee Stain: Placebo-controlled, Double-blind *In Situ* Examination

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Clinical Relevance

Chewing gum containing sodium metaphosphate is useful for whitening after eating colored food.

SUMMARY

This study aimed to evaluate the ability of chewing gum containing sodium metaphosphate (SMP) to remove coffee stains from enamel *in situ*. This was a double-blind (subjects, evaluators), parallel-group, crossover, randomized clinical trial with 30 healthy adult volunteers. Each participant held an appliance with a hydroxyapatite (HA) pellet on the lower

lingual side of his or her mouth for two hours to allow pellicle formation. The appliances were subsequently immersed in coffee solution at 37°C for 48 hours. The color of the HA pellet before and after coffee immersion was measured using a spectrophotometer. The participant set the appliance and chewed two pieces of test gum, which contained 7.5 mg of SMP per piece, or control gum without SMP. Each cycle included five minutes of exposure to chewing

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gum, after which the appliances were placed in 100% relative humidity at room temperature for a 30-minute incubation. This cycle was repeated five times for each gum type. The color of the HA pellet was measured after each chewing cycle using the spectrophotometer. In addition, ΔE^* values, which indicate the change in pellet color after each chewing cycle compared with after coffee immersion, were calculated. Data were analyzed using the paired *t*-test with Bonferroni adjustment to compare ΔE^* values of control and test gum after each chewing cycle. The ΔE^* values of test gum were significantly higher than those of control gum after all chewing cycles, excluding the first cycle ($p < 0.05$). This finding indicates that test gum containing SMP was more effective at removing coffee stains from the HA pellet than control gum. We conclude that chewing gum containing SMP can effectively remove coffee stains from HA pellets. Thus, SMP is a promising agent to be further explored in tooth-cleaning studies.

INTRODUCTION

The teeth are always seen during conversation, and a smile furnished with a set of beautiful, white teeth positively adds to a person's overall appearance. The formation and accumulation of dental stains have become some of the most important esthetic problems for many patients. Therefore, bleaching, stain removal, or other therapies that whiten the teeth are crucial concerns. The deposition of a coloring substance from coffee, red wine, smoking, and some foods and drinks onto the dental pellicle corresponds to an extrinsic dental stain. It can be difficult to avoid such staining, even when maintaining good oral hygiene. Therefore, both physical and chemical methods to remove the stain are often needed to improve such situations.¹

Hydrogen peroxide and urea peroxide have been commonly used in professional tooth bleaching.^{2,3} However, they are associated with adverse side effects or complications.⁴⁻⁶ As peroxides are known to damage the teeth and cause hyperesthesia at high concentrations, they have not been approved for home use or as an addition to dentifrices by the Japanese Ministry of Health, Labour, and Welfare from a safety viewpoint.

In contrast, condensed phosphate, which is used as a component of many dentifrices and a food additive, has been shown to safely exert a whitening effect on the tooth.⁷ Sodium metaphosphate (SMP) has a

number of uses. It is commonly applied as a food additive owing to its water-retentive, buffering, and washing actions. It is also used in cosmetics and as a quasi-drug in medicine to maintain the blood concentration of antibiotics. Recent studies reported that SMP can prevent periodontal disease and caries and enhance bone formation.⁸⁻¹³ SMP has a potential high affinity for hydroxyapatite (HA) and may exhibit stronger binding to the tooth than a stain. Thus, it is considered that SMP competes with the stain to bind to HA, and the stain is removed from the tooth during this process. Since chewing gum is well accepted by both adults and children and can be frequently used with ease, SMP-containing chewing gum may aid in removing dental stains.

Therefore, the purpose of this study was to evaluate the efficacy of a chewing gum containing SMP in removing coffee stains from HA pellets in a double-blind *in situ* clinical trial. The null hypothesis tested was that there is no difference in the ability to remove coffee stains between gum containing SMP and control gum without SMP.

METHODS AND MATERIALS

Participants

This study was a double-blind (subjects, evaluators), parallel-group, randomized clinical trial including adult volunteers. Volunteers were recruited at the hospital associated with our university (staff and students). The study was approved by the university's ethics oversight committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (reference 8/2014). Informed consent was obtained from all participants included in the study. Inclusion and exclusion criteria are indicated in Table 1. The participants were between 26 and 58 years old. All selected subjects agreed to refrain from using whitening products or procedures and to avoid the use of any dentifrices and toothbrushes other than those assigned for use during the study. The participants were also free from oral diseases or diseases with oral symptoms and did not use prostheses and orthodontic appliances. Moreover, they had the required number of teeth to retain the appliances used in this study and were not sensitive to SMP-containing chewing gum. In addition, they were available for the total five-day experimental period, able to chew gum five times per day and able to comply with the rules and instructions of the study. The participants were not pregnant or lactating women, were not smokers and did not use dentifrices for tooth whitening or chlorhexidine for

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria		
Willing to sign a consent form		
Between 26 and 58 years old		
Free from any disease with oral effects		
Has sufficient teeth to support an intraoral device (appliance)		
Available for a total of five days for the experiment and able to chew gum five times per day		
Able to comply with the rules and instructions of the study		
Exclusion Criteria		
Gross pathology in the oral cavity		
Presence of oral prostheses or orthodontic appliances		
Underwent or undergoing surgery for periodontal disease		
Pregnant or lactating woman		
Smoker		
Using dentifrices for tooth whitening and chlorhexidine for gargling		
Dry mouth		
Food and metal allergies		
Eating disorder		

gargling. All participants received the same supply of a toothbrush and a dentifrice (Clear Clean, KAO, Tokyo, Japan) without SMP. They were asked to use them from a week before the start of this trial until the end of this trial.

Chewing Gum Tested

Test gum containing SMP and control gum without SMP (Lotte, Tokyo, Japan) were used and put in bags marked "P" or "Q." All individuals involved in the experiment, except for the manufacturer, were not informed as to which bag contained test gum and which bag contained control gum until the end of the study. The composition of the chewing gum is indicated in Table 2. Test gum contained 7.5 mg SMP per piece as an additional ingredient; otherwise, it was identical to control gum in taste, shape, weight, and color. Participants were given gum labeled "P" or "Q" and were blinded to the experiment until all data were collected for analysis.

Table 2: Gum Composition (One Piece)

Components	Control Gum (% Weight)	Test Gum (% Weight)
Xylitol	39	39
Maltitol	35	35
Gum base	20	20
Sodium metaphosphate	0	0.5
Essence	3	3
Others	3	2.5

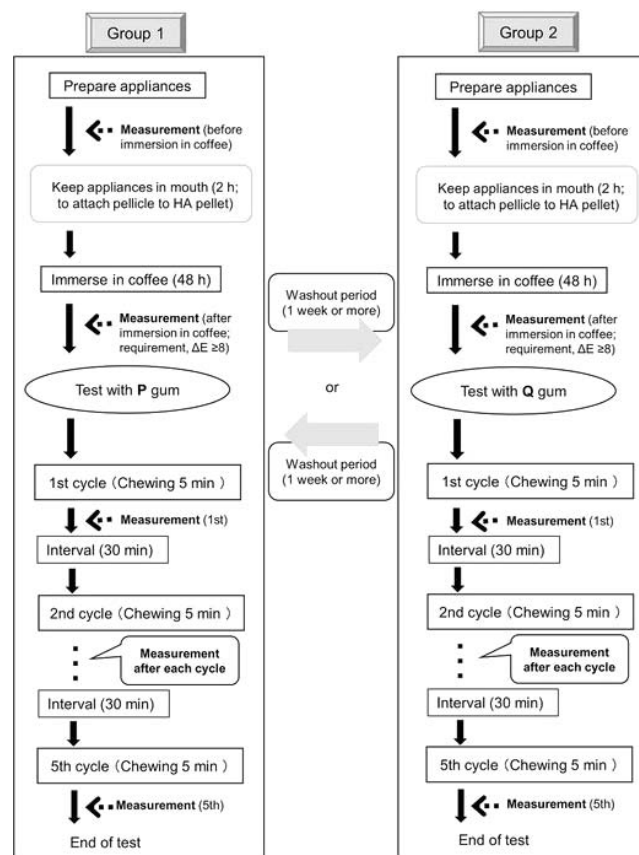


Figure 1. Experimental design.

Appliance

Impressions were taken from the mandible of all participants, and appliances were made on cast models. An HA pellet (10×10×2 mm; APP-100, Cosmo Bio, Tokyo, Japan) was attached to the lower lingual side of each appliance with resin (Palapress vario, Heraeus Kulzer, South Bend, IN, USA).¹⁴ Before attachment, 4-META/MMA-TBB resin (opaque ivory; Super Bond, Sun Medical, Shiga, Japan) was applied on the back of the pellet to ensure uniform visibility of color beneath the pellet. Then, wires ($\phi=0.9$ mm) were used to hold the appliances on teeth. Each participant was supplied with two appliances (one for each chewing gum). The surface of the HA pellet was polished using #400 waterproof silicon carbide paper (Sankyo Chemical, Saitama, Japan).

Protocol and Color Measurement

We adopted a crossover design protocol. The flow-chart of the experimental process is shown in Figure 1. Thirty participants (10 women and 20 men) between the ages of 26 and 58 years (mean age, 31 years) were equally and randomly distributed into

groups 1 and 2. The method used to generate the random allocation sequence was simple randomization. Group 1 participants used P gum first and then Q gum after a ≥ 1 -week washout period, while group 2 participants used Q gum first and then P gum after a ≥ 1 -week washout period.

The color of HA pellets before coffee immersion was evaluated using a spectrophotometer (CM 2600d, Konica Minolta, Osaka, Japan) with the CIE $L^*a^*b^*$ color space system. The conditions of color evaluation were as follows: D65 standard light, specular component included, and 2° observed angle. All participants kept the appliances in their mouth for two hours to form a pellicle on the HA pellets. The appliances were then immersed in 1.4% coffee solution (AGF, Maxim, Tokyo, Japan.) at 37°C for 48 hours and the color of HA pellets was again measured. The ΔL^* , Δa^* , Δb^* , and ΔE^* values (before and after coffee immersion) were calculated, and pellets with ΔE^* values < 8.0 were excluded from the experiment.

After coffee immersion, the participants kept the appliance in their mouth and were asked to chew on the molar side of the appliance without consuming food or drink during the five-minute chewing cycle. After the chewing cycle, the CIE $L^*a^*b^*$ value was estimated again. The appliances were then placed in 100% relative humidity at room temperature for 30 minutes. This cycle was repeated five times for each gum type. After the fifth measurement, a sufficient washout period was given to allow for the elimination of the influence of SMP (one week or more). Thereafter, a new experimental row with a new appliance and different chewing gum type was conducted (Figure 1).

To evaluate cleaning efficacy of test and control gum, ΔL^* , Δa^* , Δb^* , and ΔE^* were calculated from the color of the pellets after immersion in coffee and after each chewing cycle as follows:

$$\Delta L^* = L^*_{(\text{each chewing cycle})} - L^*_{(\text{after coffee immersion})}$$

$$\Delta a^* = a^*_{(\text{each chewing cycle})} - a^*_{(\text{after coffee immersion})}$$

$$\Delta b^* = b^*_{(\text{each chewing cycle})} - b^*_{(\text{after coffee immersion})}$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Statistical Analysis

All data (ΔL^* , Δa^* , Δb^* , and ΔE^*) were statistically analyzed using the paired *t*-test with Bonferroni

adjustment to compare control gum with test gum after each chewing cycle. Statistical analyses were performed using IBM SPSS Statistics, Version 20.0 (IBM Japan, Ltd, Tokyo, Japan).

RESULTS

Participants

All participants completed the study and none reported health problems linked to SMP exposure.

Color Change by Coffee Immersion

Color change (ΔL^* , Δa^* , Δb^*) by coffee immersion was -5.0 , 0.1 , and 9.1 for test gum and -4.7 , 0.1 , and 8.6 for control gum, respectively. Hence, the color of the HA pellet became darker and more yellowish, though the amount of redness showed almost no change following coffee immersion. The shift in color differences (ΔE^*) by coffee immersion was > 8.0 in all subjects. The mean ($\pm$ standard deviation) ΔE^* values of test and control gum were $10.4 (\pm 1.7)$ and $9.8 (\pm 1.4)$, respectively, which were not statistically significant (paired *t*-test: $p > 0.05$). Hence, all HA pellets were sufficiently stained.

Change of ΔL^* , Δa^* , and Δb^* by Gum Chewing

Figure 2 shows mean ΔL^* , Δa^* , and Δb^* values after chewing both gum types in comparison with the color coordinates of HA pellets after coffee immersion. The change in ΔL^* values after chewing both gum types showed a tendency to increase with the number of chewing cycles. In contrast, the change in Δb^* values after chewing both gum types indicated a tendency to decrease with the number of chewing cycles. Δa^* values after chewing both gum types remained relatively unchanged. Comparisons between test and control gum demonstrated ΔL^* values of test gum were significantly higher than those of control gum after all chewing cycles ($p < 0.05$), whereas Δb^* values of test gum were significantly lower than those of control gum after the third and fourth chewing cycles ($p < 0.05$). However, Δa^* values were not significantly different between gum types for all chewing cycles.

Change of ΔE^* by Gum Chewing

The change in ΔE^* values after chewing each gum type is shown in Figure 3. For control gum, mean ΔE^* values between baseline (after immersion in coffee) and after each chewing cycle were 2.6 , 3.2 , 3.5 , 3.7 , and 3.8 , respectively. For test gum, mean ΔE^* values were 3.2 , 4.1 , 4.5 , 4.8 , and 4.9 , respectively. Comparisons between test and control gum

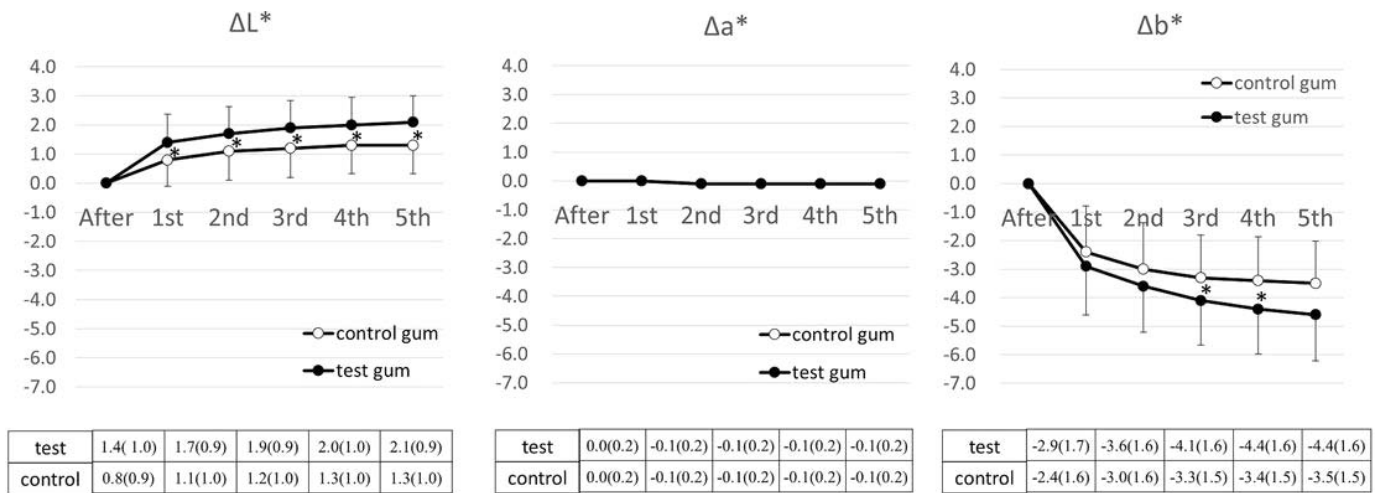


Figure 2. Change of ΔL^* , Δa^* , and Δb^* by gum chewing. Mean ΔL^* , Δa^* , and Δb^* values after chewing both gum types are shown in comparison with the color coordinates of HA pellets after coffee immersion. Asterisks (*) at each chewing cycle indicate a significant difference between control and test gum ($p < 0.05$). ΔL^* , Δa^* , and Δb^* values at each chewing cycle are also given in tables below the graphs. The values in parentheses indicate standard deviation.

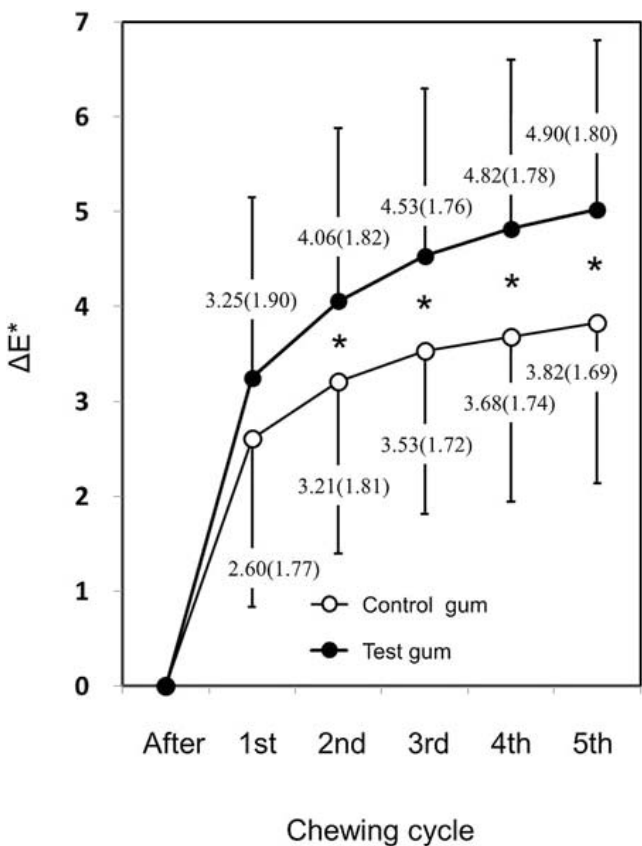


Figure 3. Change of ΔE^* by gum chewing. The ΔE^* values of control and test gum after each chewing cycle are shown in comparison with the color of HA pellets after coffee immersion. Asterisks (*) at each chewing cycle indicate a significant difference between control and test gum ($p < 0.05$). The values in parentheses indicate standard deviation.

after each chewing cycle indicated that ΔE^* values of test gum were significantly higher than those of control gum after all chewing cycles, excluding the 1st cycle ($p < 0.05$).

DISCUSSION

To our knowledge, this is the first double-blind clinical study to compare the efficacy of gum containing SMP and SMP-free gum to reduce dental stains. The chemical formula of metaphosphate is expressed by the general formula for condensed phosphates, $M_n^I(PO_3)_n$. A condensed phosphate is chemically produced by heating orthophosphoric acids (H_3PO_4) and is classified by structure into polyphosphate, metaphosphate, and ultraphosphate. SMP is listed as a safe food additive by the US Food and Drug Administration and as a safe cosmetic additive by the US Cosmetic Ingredient Review. SMP is used as a food additive to retain moisture and maintain food shape and texture. In addition, condensed phosphate has recently been used for stain removal.¹⁵ Adding condensed phosphate to a dentifrice is safe and convenient to use. In this study, stain removal efficacy of SMP was evaluated using chewing gum.

The result of our clinical study revealed a decreasing L^* value and increasing b^* value after immersing the appliance in coffee solution, though the a^* value showed almost no change. The HA pellet changed in color from nearly white to yellowish and grayish color during the immersion process. Interestingly, we found that the L^* value tended to increase and the b^* value tended to decrease, though

the a^* value remained almost unchanged, after chewing test gum containing SMP. This finding suggests that the color shift from grayish and yellowish color to white occurred before coffee immersion, to some extent. Regarding ΔE^* , the mechanism of color change clearly increased after each subsequent cycle until the fifth chewing cycle, and ΔE^* values of test gum were significantly higher than those of control gum after all chewing cycles except for the first cycle ($p < 0.05$; Figure 3). One potential explanation for this finding may be different chemical and molecular mechanisms of the destaining action. With control gum, the stain was cleaned to some degree by rinsing with saliva and friction with soft tissue, such as the tongue and gum itself. However, with test gum, chemical destaining likely occurred in addition to the rinsing and mechanical cleaning effect of mastication. Because SMP was the only component that differed in the composition of control and test gum, the difference in ΔE^* values between control and test gum after each chewing cycle must be derived from the chemical destaining effect of SMP. Therefore, the null hypothesis—there is no difference in the efficacy of removing coffee stains between gum containing SMP and control gum without SMP—was rejected. Therefore, chewing gum containing SMP appears to be effective at removing coffee stains, which have high acidity and easily permeate the enamel.^{2,16-19}

Several studies have evaluated the use of condensed phosphates for decreasing stain formation or stain removal.²⁰⁻²² Baig and others²⁰ stated that the effect of sodium hexametaphosphate is likely to decrease stain formation due to the higher affinity of sodium hexametaphosphate to tooth calcium ions than to tea chromogens. In addition, they showed that sodium hexametaphosphate can prevent deposition and decrease staining using HA pellet powder as a substrate to adsorb tea chromogens. They discuss that binding of the phosphate group to calcium on the enamel surface is the thermodynamic affinity of enamel apatite. The adsorbed pellicle protein is removed while the condensed phosphate is simultaneously adsorbed. In other words, the stain adsorbed on the surface of enamel apatite or stain attached to the surface of the pellicle is replaced by condensed phosphate.²⁰ Thus, the whitening effect of SMP observed in our study might be explained by a higher affinity between condensed phosphate and HA pellets than between the stain and HA pellets.

Koyasu and others²¹ suggested that ultraphosphate binds to the glass ionomer cement slice and can replace stains that are bound to its surface. They

investigated the influence of temperature on stain removal by ultraphosphate and found significantly greater stain removal at 37°C than 20°C.²¹ In the present study, test gum containing SMP was kept in the human oral cavity, which is approximately 36°C to 37°C. We speculate that the higher temperature of the oral cavity in comparison with room temperature enhanced the chemical action of SMP, thereby resulting in the significant difference in stain removal between test and control gum.

In addition, Porciani and others²² conducted a double-blind clinical study of chewing gum containing a condensed phosphate as a cleaning agent. Participants chewed gum with or without 1% sodium tripolyphosphate for 10 min/d for six weeks to remove stains. The authors observed decreased staining following the use of chewing gum containing the tripolyphosphate.²² In the present study, there was a significant difference even within a short period of five times a day for five minutes. Thus, the stain removal ability of SMP may have further increased over time. It is not clear whether there is a difference in the stain removal effect of SMP according to the period of SMP activity and the properties of coloring substances. It is difficult to simulate mastication to investigate stain removal ability in an *in vitro* study. Therefore, additional clinical studies are necessary. Further studies are also needed to evaluate the effect of acidity, hydrophobicity, or solubility on the stain removal ability of SMP and differences according to period of SMP activity.

CONCLUSIONS

Chewing gum containing SMP can effectively remove coffee stains from HA pellets. Thus, SMP is a promising agent to be further explored in tooth whitening studies.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Hokkaido University Dental Hospital. The approval code for this study is: # 8/ 2014.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Bonding Performance of Simplified Adhesive Systems in Noncarious Cervical Lesions at 2-year Follow-up: A Double-blind Randomized Clinical Trial

RF Zanatta • TM Silva • MALR Esper • E Bresciani • SEP Gonçalves • TMF Caneppele

Clinical Relevance

The composition and adhesion strategy of current simplified adhesives may be used for noncarious cervical lesions with minimal dentin sclerosis.

SUMMARY

Objectives: This study aimed to evaluate the bonding performance of a universal adhesive used according to self-etching or etch-and-rinse protocols in noncarious cervical lesions (NCCLs) and to compare the two protocols with their respective gold standard techniques.

Methods and Materials: This randomized, double-blind clinical study enrolled 34 partici-

pants who met the inclusion criteria, 29 of whom returned after two years. They received 152 restorations bonded with one of the three adhesives (Scotchbond Universal Adhesive, Adper Single Bond 2, or Clearfil SE Bond) and one of the two bonding techniques tested. The NCCLs were restored with nanocomposite resin (Filtek Supreme). Final contours were done with a fine diamond rotary instrument and polished with rubber points. The restorations were evaluated using the FDI World

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Dental Federation criteria at baseline (seven days after the restoration procedure), 6, 12, and 24 months. Descriptive statistics, Kruskal-Wallis, Friedman analysis of variance, and least significant difference tests were performed.

Results: No statistically significant differences were found in esthetics or the functional and biological evaluated criteria among the adhesive systems and techniques.

Conclusions: The Scotchbond Universal system behaved similarly to the conventional etch-and-rinse or self-etching systems in all the adhesion strategies in the evaluated periods.

INTRODUCTION

The development of new adhesive systems has been prompted by the need to simplify single-bottle adhesives and provide faster, more user-friendly, and less technique-sensitive products.¹ Recently, multimode simplified adhesive systems with fewer steps, known as universal adhesives, have been launched as viable options for enamel and dentin bonding.^{2,3} These adhesives can be used with the self-etching or etch-and-rinse strategy^{3,4} and act as silane agents for ceramics and metals.⁵

Differences in composition and structure between enamel and dentin make the bonding strategy decision challenging. The etch-and-rinse strategy with phosphoric acid remains the gold standard for enamel. Studies on self-etching adhesives^{6,7} indicate that the adhesives are usually not able to form a proper and uniform layer, resulting in lower bond strengths compared with etch-and-rinse systems.⁸ Dentin, however, has a higher organic content than enamel, and etching with phosphoric acid can

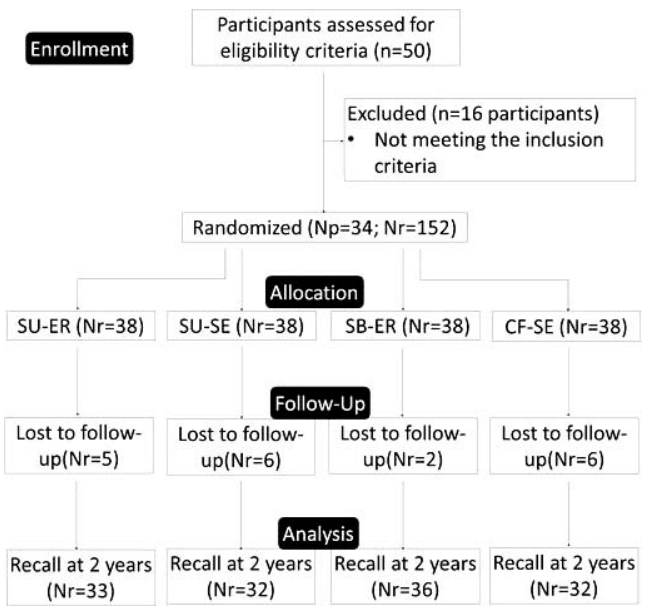


Figure 1. Flowchart of the Model Under Study. Np, number of participants; Nr, number of restorations; SU, Scotchbond Universal Adhesive; ER, etch-and-rinse; SE, self-etching; SB, Adper Single Bond 2; CF, Clearfil SE Bond.

increase collagen degradation by activating metalloproteinases and cathepsins. The use of self-etching adhesives could decrease this degradation phenomenon.⁹⁻¹¹

Recent studies with universal multimode adhesives have shown that the bonding strategy used—self-etching or etch-and-rinse—does not influence short-term bonding performance.^{3,12,13} However, at a three-year follow-up, some marginal degradation was found for the self-etching strategy when used in noncarious cervical lesions (NCCLs).¹⁴

NCCLs are characterized by cervical dental tissue loss without the presence of caries. Their etiology is multifactorial and is often associated with dentin

Table 1: Dentin Sclerosis ^a and Preoperative Sensitivity Scales	
Category	Criteria
Dentin Sclerosis Scale	
1	No sclerosis present; dentin is light yellowish or whitish with little discoloration; dentin is opaque with little translucency or transparency
2	More sclerosis than category 1 but less than halfway between categories 1 and 4
3	Less sclerosis than category 4 but more than halfway between categories 1 and 4
4	Significant sclerosis present; dentin is dark yellow or even discolored (brownish); glassy appearance with significant translucency or transparency evident
Preoperative Sensitivity Scale	
1	Absence of dentin sensitivity
2	Presence of dentin sensitivity

^a Adapted from Swift et al.¹⁸

Table 2: Compositions and Application Mode of the Adhesive Systems			
Adhesive System	Composition		Application Mode ^a
Scotchbond Universal Adhesive (3M ESPE, St Paul, MN, USA)	Methacryloyloxydecyl dihydrogen phosphate, phosphate monomer, dimethacrylate resins, hydroxyethyl methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane.	Etch-and-rinse	Apply etchant for 15 s.
			Apply the adhesive for 20 s,
			Gently air for 5 s,
			Light cure for 10 s.
		Self-etch	Do not etch.
			Apply the adhesive for 20 s,
Gently air for 5 s,			
			Light cure for 10 s.
Adper Single Bond 2 (3M ESPE, St Paul, MN, USA)	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiators, methacrylate functional copolymer of polyacrylic, polyitaconic acids, silica particles.	Etch-and-rinse	Apply etchant for 15 s.
			Apply 2 to 3 consecutive coats of the adhesive for 15 s,
			Gently air for 5 s,
			Light cure for 10 s.
Clearfil SE Bond (Kuraray, Kurashiki, Okayama, Japan)	<i>Primer:</i> 10-MDP, HEMA, hydrophilic dimethacrylate, di-camphorquinone, N,N-diethanol-p-toluidine, water. <i>Bond:</i> 10-MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, di-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica	Self-etch	Do not etch.
			Apply primer and leave for 20 s,
			Do not rinse,
			Dry with mild air flow,
			Apply the adhesive and distribute with mild air flow,
			Light cure for 10 s.
Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol A-glycidyl methacrylate.			
^a According to the manufacturer's instructions.			

sensitivity and poor esthetics.¹⁵ Bonding to NCCLs is challenging because of the general absence of macromechanical retention and the presence of both enamel and dentin margins requiring different adhesive strategies. Therefore, this clinical study aimed to evaluate the bonding performance of a universal adhesive used according to the self-etching and the etch-and-rinse protocols in NCCLs and to compare the two protocols to their respective gold standard techniques. The null hypothesis tested was that no difference would be found between the two protocols after a two-year follow-up.

METHODS AND MATERIALS

This randomized, double-blind clinical study followed the CONSORT statement.¹⁶ The experimental design was a controlled, double-blind, randomized clinical trial and was conducted after approval from the local Institutional Review Board. This trial was registered in the Brazilian Clinical Trials Registry (ReBEC - www.ensaiosclinicos.gov.br) under the identification number RBR-4rw55d.

Sample-Size Calculation

The tooth was used as the statistical unit. Sealed Envelope online software (www.sealedenvelope.com) was used to calculate the sample size. At least 30 teeth per group were required to provide an 80%

assurance that the limits of a two-sided 90% confidence interval would exclude a difference among the groups of more than 15%. To account for dropout during the experimental period, an enrollment of 38 teeth per group was determined.

Participants

Fifty participants from the Department of Restorative Dentistry triage center were examined by four precalibrated operative dentistry professionals to determine whether they met the inclusion criteria (Figure 1). Teeth with NCCLs were selected by using a mouth mirror, an explorer, and a periodontal probe. Prior to participation in the study, all participants signed a written informed consent and received oral hygiene instructions.

The study inclusion criteria were that the patient had to be in good general health, be at least 18 years old, have an acceptable oral hygiene level, and present with at least 20 teeth in occlusion. Participants were required to exhibit NCCLs with a depth of at least 1 mm and have both enamel and dentin margins with the cavosurface margin of at least 50% enamel. Also, the selected teeth had to be vital and not mobile.^{12,13,17} Individuals with poor oral hygiene, severe bruxism, and severe or chronic periodontitis were excluded.

Table 3: FDI Criteria Used for Clinical Evaluation²⁰

	Esthetic Property	Functional Properties		Biological Properties	
	1. Marginal Staining	2. Fracture and Retention	3. Marginal Adaptation	4. Postoperative Sensitivity	5. Secondary Caries
1. Clinically very good	1.1 No marginal staining	2.1 Restoration retained, no fractures/ cracks	3.1 Harmonious outline, no gaps, no discoloration	4.1 No hypersensitivity	5.1 No secondary or primary caries
2. Clinically good (after correction very good)	1.2 Minor marginal staining, easily removable by polishing	2.2 Small hairline crack	3.2.1 Marginal gap (50 µm) 3.2.2 Small marginal fracture removable by polishing	4.2 Low hypersensitivity for a limited period of time	5.2 Very small and localized demineralization. No operative treatment required
3. Clinically sufficient/ satisfactory (minor shortcoming with no adverse effects but not adjustable without damage to the tooth)	1.3 Moderate marginal staining, not esthetically unacceptable	2.3 Two or more or larger hairline cracks and/or chipping (not affecting the marginal integrity)	3.3.1 Gap >150 µm not removable 3.3.2 Several small enamel or dentin fractures	4.3.1 Premature/ slightly more intense. 4.3.2 Delayed/ weak sensitivity; no subjective complaints, no treatment needed	5.3 Larger areas of demineralization, but only preventive measures necessary (dentin not exposed)
4. Clinically unsatisfactory (repair for prophylactic reasons)	1.4 Pronounced marginal staining; major intervention necessary for improvement	2.4 Chipping fractures that damage marginal quality; bulk fractures with or without partial loss (less than half of the restoration)	3.4.1 Gap >250 µm or dentin/base exposed 3.4.2 Chip fracture damaging margins 3.4.3 Notable enamel or dentin wall fracture	4.4.1 Premature/ very intense 4.4.2 Extremely delayed/weak with subjective complaints 4.4.3 Negative sensitivity; intervention necessary but not replacement	5.4 Caries with cavitation (localized and accessible and can be repaired)
5. Clinically poor (replacement necessary)	1.5 Deep marginal staining not accessible for intervention	2.5 Partial or complete loss of restoration	3.5 Filling is loose but <i>in situ</i>	4.5 Very intense, acute pulpitis or nonvital Endodontic treatment is necessary, and restoration has to be replaced	5.5 Deep secondary caries or exposed dentin that is not accessible for repair of restoration

Restorative Procedures

Before placement of the restorations, the characteristics of the NCCLs were evaluated. The cavity dimensions in millimeters (height, width, and depth) and the geometry of the cavity (labeled at <90°, 90° to 135°, >135°) were evaluated from a photographic profile. Each profile was measured by computer software (Image J v1.52, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The amount of sclerotic dentin was measured according to the criteria described by Swift and others¹⁸ (Table 1). Preoperative sensitivity was also evaluated by applying air for 10 seconds from a dental syringe placed 2 cm from the tooth surface (Table 1).

Participants who met the inclusion criteria received restorations bonded with one of the three adhesives and one of the two bonding techniques tested. Randomization was achieved by using a random list generated by a website (www.random.

org) and allocated using sealed envelopes. The operators were not blinded to group assignment when performing the restorations; however, participants and examiners were blinded to the adhesive and technique used.

Treatment was performed according to a predetermined procedure, which included prophylaxis of the cavities using a rubber cup with pumice and water; shade selection with VITA Classical shade guide, and local anesthesia (Mepivalem AD 2%, DLA Pharmaceutical Ltda, Dentsply, Catanduva, SP, Brazil). No additional retention or bevels were made in the cavities. All procedures were carried out using the relative isolation method with cotton rolls, saliva aspirator, and gingival retraction cord (Ultrapak #000, Ultradent Products, Inc, South Jordan, UT, USA) in the gingival sulcus.

The teeth were etched with 37% phosphoric acid (Condac 37, FGM, Joinville, SC, Brazil) applied for

Table 4: *Baseline Characteristics of the Subjects and the Noncarious Cervical Lesions Included in the Study Groups*

Characteristics of Research Participants		No. (%) of Subjects/Lesions			
Sex					
Male		13 (38)			
Female		21 (62)			
Age (y)					
< 20		0 (0)			
21-40		5 (15)			
41-60		27 (79)			
> 60		2 (6)			
Characteristics of Class V lesions		SU/ER	SU/SE	SB/ER	CF/SE
Degree of dentin sclerosis					
1	28 (18.4)	24 (15.8)	24 (15.8)	24 (15.8)	
2	9 (5.8)	12 (7.8)	13 (8.6)	13 (8.6)	
3	1 (0.7)	2 (1.3)	0 (0)	1 (0.7)	
4	0 (0)	0 (0)	1 (0.7)	0 (0)	
Tooth distribution					
Anterior					
Incisors	4 (2.6)	5 (3.3)	4 (2.6)	4 (2.6)	
Canines	3 (2.0)	4 (2.6)	7 (4.6)	3 (2.0)	
Posterior					
Premolar	22 (14.5)	22 (14.5)	21 (13.8)	24 (15.8)	
Molar	9 (5.9)	7 (4.6)	6 (3.9)	7 (4.6)	
Position					
Maxillary	22 (14.5)	21 (13.8)	22 (14.5)	23 (15.1)	
Mandibular	16 (10.5)	17 (11.2)	16 (10.5)	15 (9.9)	
Depth					
Flat (1.0-1.5 mm)	11 (7.2)	10 (6.6)	9 (5.9)	9 (5.9)	
Medium (1.5-2.0 mm)	17 (11.2)	21 (13.8)	16 (10.5)	16 (10.5)	
Deep (>2.0 mm)	10 (6.6)	7 (4.6)	13 (8.6)	13 (8.6)	
Cervico-incisal height (mm)					
<1.5	6 (3.9)	6 (3.9)	6 (3.9)	5 (3.3)	
1.5-2.5	19 (12.5)	22 (14.5)	18 (11.8)	22 (14.5)	
2.6- 4.0	9 (5.9)	7 (4.6)	8 (5.3)	5 (3.3)	
>4.0	4 (2.6)	3 (2.0)	6 (3.9)	6 (3.9)	
Preoperative sensitivity					
Yes	18 (11.8)	24 (15.8)	19 (12.5)	20 (13.2)	
No	20 (13.2)	14 (9.2)	19 (12.5)	18 (11.8)	
Width (mm)					
< 4.0	21 (13.8)	19 (12.5)	21 (13.8)	22 (14.5)	
4.0- 5.9	11 (7.2)	16 (10.5)	14 (9.2)	11 (7.2)	
6.0- 7.9	3 (2.0)	2 (1.3)	3 (2.0)	4 (2.6)	
> 8.0	3 (2.0)	1 (0.7)	0 (0.0)	1 (0.7)	
Shape of angle					
< 90	6 (3.9)	5 (3.3)	7 (4.6)	3 (2.0)	
90-135	26 (17.1)	29 (19.1)	27 (17.8)	32 (21.1)	
> 135	6 (3.9)	4 (2.6)	4 (2.6)	3 (2.0)	
Abbreviations: SU, Scotchbond Universal Adhesive; ER, etch-and-rinse; SE, self-etch; SB, Adper Single Bond 2; CF, Clearfil SE Bond.					

Table 5: Number and Percentage of Success for Each Studied Variable (Kruskal-Wallis)

Variable	Success (Scores 1, 2, and 3) No. of restorations (%)	Failure (scores 4 and 5) No. of restorations (%)	p-value
Degree of dentin sclerosis			
1	67 (50.4)	15 (11.3)	0.5135
2	40 (30.1)	6 (4.5)	
3	3 (2.3)	1 (0.8)	
4	1 (0.8)	0 (0.0)	
Depth			
Flat (1.0-1.5mm)	26 (19.5)	5 (3.8)	0.1603
Medium (1.5-2.0mm)	56 (42.1)	7 (5.3)	
Deep (>2.0mm)	29 (21.8)	10 (7.5)	
Cervico-incisal height (mm)			
<1.5	11 (8.3)	4 (3.0)	0.5034
1.5- 2.5	62 (46.6)	9 (6.8)	
2.6- 4.0	22 (16.5)	6 (4.5)	
>4.0	16 (12.0)	3 (2.3)	
Width (mm)			
< 4.0	59 (44.4)	10 (7.5)	0.6083
4.0- 5.9	40 (30.1)	10 (7.5)	
6.0- 7.9	7 (5.3)	2 (1.5)	
> 8.0	5 (3.8)	0 (0.0)	
Shape of angle			
< 90	13 (9.8)	4 (3.0)	0.1362
90- 135	87 (65.4)	13 (9.8)	
> 135	11 (8.3)	5 (3.8)	

30 s to the enamel and 15 seconds to the dentin (depending on the technique used), followed by air-water spray for 30 seconds to remove the acid. Excess water was removed with absorbent paper, leaving a moist dentin surface. Subsequently, the cavities received the adhesive system applied using the two different techniques, etch-and-rinse or self-etch, according to the manufacturer's instructions. The compositions and application mode are described in Table 2.

The cavities were restored with a direct restorative nanocomposite resin (Filtek Supreme, 3M ESPE, St Paul, MN, USA). Increments up to 2 mm in thickness were placed and light polymerized (750 mW/cm²; Emitter A, Guilin Woodpecker Medical Instrument Co, Guilin, China) for 20 seconds each. Final contour was achieved with a fine diamond rotary instrument (KG Sorensen, SP, Brazil) immediately after the restorative procedure. The restorations were polished with rubber points (Astropol F, Ivoclar Vivadent AG, SP, Brazil) seven days after placement.

Calibration Procedures for Clinical Assessment

For training purposes, two experienced and calibrated examiners observed 10 photographs that were representative of each score for each criterion. They evaluated 10 individuals each on two consecutive days. These individuals had cervical restorations but were not part of this study. An intraexaminer and interexaminer agreement of at least 85% was necessary before beginning the evaluation.

Clinical Assessment

The restorations were evaluated using the FDI World Dental Federation criteria^{19,20} as shown in Table 3. The most relevant items for testing the adhesive performance were selected. The primary measurable variable was restoration retention and fractures, followed by the secondary measurable variables: marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries. Those measurable variables were ranked as (1) clinically very good, (2) clinically good, (3) clinically

Table 6: Comparison of the Tested Adhesive Systems According to the FDI Criteria Compared by Kruskal-Wallis Test at $p < 0.05$ for Each Recall Time ^a

Time	Baseline (After 7 Days)					6 months				
FDI Criteria	SU/ER	SU/SE	SB/ER	CF- SE	<i>p</i> ^a	SU -ER	SU- SE	SB- ER	CF- SE	<i>p</i>
Functional properties										
Fracture and retention										
VG	38	37	38	38	0.392	36	35	37	35	0.725
GO	-	1	-	-		2	3	1	2	
SS	-	-	-	-		-	-	-	-	
UN	-	-	-	-		-	-	-	-	
PO	-	-	-	-		-	-	-	1	
Marginal adaptation										
VG	38	37	37	37	0.799	36	33	37	33	0.301
GO	-	1	1	1		2	5	1	3	
SS	-	-	-	-		-	-	1		
UN	-	-	-	-		-	-	-		
PO	-	-	-	-		-	-	-		
Esthetics properties										
Marginal staining										
VG	38	38	38	38	-	36	36	35	32	0.512
GO	-	-	-	-		2	1	3	4	
SS	-	-	-	-		-	1	-	1	
UN	-	-	-	-		-	-	-		
PO	-	-	-	-		-	-	-	-	
Biological properties										
Postoperative sensitivity										
VG	35	34	34	36	0.806	34	33	35	33	0.910
GO	3	3	3	2		4	4	2	4	
SS	-	-	-	-		-	1	-	-	
UN	-	1	1	-		-	-	1	-	
PO	-	-	-	-		-	-	-	-	
Recurrence of caries										
VG	38	38	38	38	-	38	38	38	37	-
GO	-	-	-	-		-	-	-	-	
SS	-	-	-	-		-	-	-	-	
UN	-	-	-	-		-	-	-	-	
PO	-	-	-	-		-	-	-	-	
Abbreviations: SU, Scotchbond Universal Adhesive; ER, etch-and-rinse; SE, self-etch; SB, Adper Single Bond 2; CF, Clearfil SE Bond; VG, clinically very good; GO, clinically good; SS, clinically sufficient/satisfactory; UN, clinically unsatisfactory; PO, clinically poor.										
^a Comparison among the groups at the same follow-up (Kruskal-Wallis test);										

Abbreviations: SU, Scotchbond Universal Adhesive; ER, etch-and-rinse; SE, self-etch; SB, Adper Single Bond 2; CF, Clearfil SE Bond; VG, clinically very good; GO, clinically good; SS, clinically sufficient/satisfactory; UN, clinically unsatisfactory; PO, clinically poor.

^a Comparison among the groups at the same follow-up (Kruskal-Wallis test);

sufficient/satisfactory, (4) clinically unsatisfactory, and (5) clinically poor.

The two calibrated examiners conducted the evaluations according to the periods: baseline (one week after the restorative procedures) and 6, 12, and 24 months after the restorative procedures. If there were disagreements between the two examiners in the individual analysis, they discussed the situation to reach a consensus. Both participants and examiners were unaware of the material used to perform

the restorations, accordingly resulting in a double-blind study.

Statistical Analysis

Statistical analysis was performed for each criterion in R statistical language R Studio (version 3.4.4, R Studio Team, Boston, MA, USA). Only data from the participants who attended the recall were included in the statistical evaluation in a procedure called per-protocol analysis. Descriptive statistics were used to demonstrate the frequency distribution of

Table 6: Comparison of the Tested Adhesive Systems According to the FDI Criteria Compared by Kruskal-Wallis Test at $p < 0.05$ for Each Recall Time ^a (ext.)

Time	12 months					24 Months				
FDI Criteria	SU- ER	SU- SE	SB- ER	CF- SE	<i>p</i>	SU- ER	SU- SE	SB- ER	CF- SE	<i>p</i>
Functional properties										
Fracture and retention										
VG	34	31	34	30	0.315	31	25	29	24	0.192
GO	1	3	2	3		-	3	1	2	
SS	-	1	1	1		-	-	2	1	
UN	-	-	-	-		-	-	-	-	
PO	1	2	-	1		1	2	4	3	
Marginal adaptation										
VG	30	32	32	27	0.512	25	14	22	16	0.074
GO	5	3	4	6		6	14	10	10	
SS	-	-	1	1		-	-	-	1	
UN	-	-	-	-		-	-	-	-	
PO	-	-	-	-		-	-	-	-	
Esthetics properties										
Marginal staining										
VG	30	27	31	24	0.546	26	17	24	17	0.251
GO	3	6	5	8		3	8	7	7	
SS	-	1	1	1		-	1	-	1	
UN	2	-	-	1		2	1	1	1	
PO	-	-	-	-		-	-	-	-	
Biological properties										
Postoperative sensitivity										
VG	30	30	31	33	0.351	30	24	26	24	0.287
GO	4	4	4	-		1	3	5	2	
SS	1	-	1	1		-	-	-	1	
UN	-	1	1	-		-	1	1	-	
PO	-	-	-	-		-	-	-	-	
Recurrence of caries										
VG	35	35	37	34	-	31	28	32	27	-
GO	-	-	-	-		-	-	-	-	
SS	-	-	-	-		-	-	-	-	
UN	-	-	-	-		-	-	-	-	
PO	-	-	-	-		-	-	-	-	

the lesions according to the characteristics of the lesions. The Kruskal-Wallis test was performed to analyze all evaluated criteria among the groups at each evaluation time. The difference in the performance of each group at baseline and after each recall visit (6, 12, and 24 months) was assessed by the Friedman repeated analysis of variance, followed by the least significant difference test for multiple comparisons when applicable. Cohen kappa statistics were used to test interexaminer agreement. In all statistical tests, the level of significance was set to 5%.

RESULTS

In this study, 152 restorations were placed in 34 participants, with a median of four restorations per participant (minimum one and maximum 10 restorations) and reevaluated after six months, one year, and two years. In the recall after two years, 29 participants were evaluated with 133 restorations. Details regarding the research participants and characteristics of the restored cavities are presented in Table 4.

None of the characteristics of the cavity influenced retention of the restoration (Table 5). Also, no

Table 7: Percentage of Acceptable Restorations (Scores VG, GO, and SS) for Each Recall Time According to the Experimental Group Compared by Friedman Test and Least Significant Difference Test at $p < 0.05$

Adhesive	Single Bond Universal – Etch and rinse (SU-ER)					Single Bond Universal – Self-Etch (SU-SE)				
	BL	6 mo	12 mo	24 mo	<i>p</i>	BL	6 m	12 mo	24 mo	<i>p</i>
Functional properties										
Fracture and retention	100	100	97	94	0.194	100 a	100 a	95 b	87.5 b	0.024
Marginal adaptation	100	100	100	100	N/A	100	100	100	100	N/A
Esthetics properties										
Marginal Staining	100	100	94	94	0.111	100	100	100	97	0.392
Biological properties										
Postoperative sensitivity	100	100	100	100	N/A	97	100	97	96	0.511
Recurrence of caries	100	100	100	100	N/A	100	100	100	100	N/A
Abbreviations: VG, clinically very good; GO, clinically good; SS, clinically sufficient/satisfactory; SU, Scotchbond Universal Adhesive; ER, etch-and-rinse; SE, self-etch; SB, Adper Single Bond 2; CF, Clearfil SE Bond; BL, baseline; N/A, not applicable.										
^a Groups connected by the same letter indicate not statistically significant differences among the follow-ups for the same group ($p > 0.05$).										

statistically significant differences in esthetics or functional and biological evaluated criteria were found among adhesive systems and techniques (Table 6).

The comparisons of each group at baseline and after each recall visit (6, 12, and 24 months) for each criterion are presented in Table 7.

DISCUSSION

The formation of NCCLs has been associated with occlusal factors²¹ and biocorrosive processes that alter dentin morphology and structure,²² directly influencing the adhesion and therefore the survival rates of these restorations. The success of restorations involving cavities without additional mechanical retention, such as NCCLs, is directly related to the adhesion strategy used.

Factors inherent to dentin, such as the presence of humidity, which varies according to the depth, presence of caries-affected tissue, degree of demineralization, and lack of retention provided by high tissue loss, have been associated with failure at the adhesive interface. Also, external factors, such as pH challenges, saliva, and thermal-mechanical stress, contribute to reduced longevity of the restorations.²³⁻²⁵ Therefore, longitudinal clinical studies are preferable in evaluating the adhesive longevity of restorations as the materials are exposed to all the oral conditions and can be tested in similar conditions.

The adhesive strategy frequently used for dentin differs from that for enamel due to the inherent characteristics of the tissues. Often, the adhesives used in dentin are self-etching and provide less aggressive acid conditioning; this minimizes the

collapse of collagen fibrils after drying, which leads to decreased bond strength.²⁶ Enamel, however, requires etching with phosphoric acid to increase retention.⁶ and currently, the so-called selective etching technique has been adopted for restoration of cavities involving both enamel and dentin. The adhesive systems chosen for this study, both etch-and-rinse and self-etching, represents the most popular systems and have been designated as gold standards in the literature. The universal system, however, was used in both adhesive strategies to evaluate its bonding performance longitudinally. The results of this study showed findings with regards to the evaluated criteria of retention, esthetics, and functional and biological factors. The null hypothesis was not rejected for a two-year follow-up of the two protocols.

The retention rates comparing the adhesion strategies and the adhesive systems used (Table 6) were not significantly different, showing that the universal system tested is a viable option for restoring NCCLs over the two-year term. However, most of the teeth restored in this study presented minimal or no dentin sclerosis. In addition, no significant differences (Table 5) were found between the success rate and the study variables (degree of dentin sclerosis, depth, height, width, angle of lesion), findings that were consistent with those of previous studies.^{13,14,27}

The action mode of universal adhesives is entirely different from that of the conventional self-etching and etch-and-rinse systems and can be applied over wet or dry dentin. The hybrid layer may not represent the primary mechanism of action in long-term dentin adhesion.²⁸ The success of universal adhesives regarding bonding is attributed to the

Table 7: Percentage of Acceptable Restorations (Scores VG, GO, and SS) for Each Recall Time According to the Experimental Group Compared by Friedman Test and Least Significant Difference Test at $p < 0.05$ (ext.)

Adhesive	Single-Bond2 – Etch and Rinse (SB-ER)					Clearfil SE – Self-Etch (CF-SE)				
	BL	6 mo	12 mo	24 mo	<i>p</i>	BL	6 mo	12 mo	24 mo	<i>p</i>
Functional properties										
Fracture and retention	100 a	100 a	100 a	89 b	0.007	100 a	97 ab	92 b	84 b	0.015
Marginal adaptation	100	100	100	100	N/A	100	100	100	100	N/A
Esthetics properties										
Marginal Staining	100	100	100	97	0.392	100	100	97	96	0.392
Biological properties										
Postoperative sensitivity	97	97	97	97	N/A	100	100	100	100	N/A
Recurrence of caries	100	100	100	100	N/A	100	100	100	100	N/A

presence of 10-methacryloyloxydecyl dihydrogen phosphate monomer, which is responsible for the chemical bonding between the adhesive and the dentin, creating a stable interface even without micromechanical retention.²⁹ This monomer also contains a phosphate radical in its molecule that, together with the carboxylate radical from the polyalkenoate copolymer in the universal adhesive, reacts with the calcium from hydroxyapatite to create stable bonds, termed “hydroxyapatite nanolayering.”^{3,29} This nanolayering is essential for bonding, not only to dentin, but also to enamel, and might explain the good performance of this adhesive on enamel even without selective etching, as shown by the results of retention and marginal adaptation and discoloration (Table 6). The pH of the universal adhesive tested is claimed to be around 2.7 to 3.0.^{14,29} This is claimed to be ultramild, and it is not strong enough to provide adequate etching of the enamel. This effective bond to enamel at two years has been previously reported.^{3,13,27,29} The retention rate and marginal adaptation have been acceptable immediately and after 18 months. However, differences between the techniques have been reported after 36 months.¹⁴

The participants in this research were predominately women, aged between 41 and 60 years old, and with dentin sclerosis category 1 (Table 4). Canines and premolars were the most affected teeth, which is consistent with a previous report.³⁰ Due to its small occlusal surface and location in the arch, the premolar undergoes load concentration during mastication. This overload can be transmitted to the cervical region of reduced enamel volume, promoting microcracks and initiating the NCCL. Also, hypersensitivity was rarely reported, the lesion height was less than 4 mm, and the lesion angle was between

90° and 135°. The data do not confirm the evolution of the lesion or a relation to patient lifestyle.

The restoration of NCCLs should include an analysis of the etiologic factors and their removal, when possible. Not removing unwanted occlusal forces on the restored teeth will also decrease the success rate, factors clinicians should be aware of when treating patients. The Single Bond Universal, in both bonding strategies used in this study, proved to be useful compared with the etch-and-rinse and self-etch conventional adhesives for a two-year period. However, more extended evaluations are necessary to establish its long-term performance.

CONCLUSION

This two-year clinical evaluation demonstrated that the universal adhesive system tested, Scotchbond Universal, performed similarly in restoring NCCLs with minimal dentin sclerosis in both strategies used compared with the etch-and-rinse (Single Bond) or self-etching (Clearfil SE Bond) systems.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the São Paulo State University. The approval code for this study is: 095374/2013.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature

or kind in any product, service, and/or company that is presented in this article.

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Laboratory Research

The Use of Composite Layering Technique to Mask a Discolored Background: Color Analysis of Masking Ability After Aging—Part II

BG Perez • LL Miotti • AH Susin • LB Durand

Clinical Relevance

The masking ability of resin composites can change over time and influence long-term performance of multilayered restorations.

SUMMARY

The aim of this study was to evaluate the effectiveness of the layering technique to mask a discolored background (C4) after one year of water aging. The technique was used with three resin composite restorative systems (IPS Empress Direct, Charisma Diamond, and Filtek Z350XT). Ninety 1.5-mm-thick specimens

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were divided into groups and subgroups according to the restorative system and resin composite shade combination used in the layering technique (enamel, body, and dentin). The color measurements were made by reflection spectrophotometer (SP60, EX-Rite) over the C4 substrate at two time points: after 24-hour water immersion (T_0) and after one-year water aging (T_1). The masking ability was evaluated by calculating the ΔE between an inherent color background and a C4 background at T_0 and T_1 . CIELAB and CIEDE2000 formulas were used for comparative analysis between T_0 and T_1 color measurements. Analysis of variance and Turkey's *post hoc* test ($\alpha=0.05$) were performed to analyze ΔE_{ab} and ΔE_{00} mean values. The Z350XT restorative system presented adequate masking ability in the combinations of 0.5 mm body + 1.0 mm dentin, 0.5 mm enamel + 1.0 mm dentin, and 1.5 mm dentin after 24-hour water immersion. The masking ability was affected negatively by water aging in most of the combinations. The IPS Empress Direct was the only system that remained stable after aging in the combinations of 0.5 mm enamel + 1.0 mm dentin and +

1.5 mm dentin ($p=0.05$ and $p=0.47$ for CIELAB; $p=0.15$ and $p=0.51$ for CIEDE2000), although it did not present adequate masking ability in both time points. None of the combinations of any system presented adequate masking ability after prolonged water aging.

INTRODUCTION

Dental discoloration compromises an individual's esthetics and smile. Bleaching is the most conservative treatment for discolored teeth.¹ However, optimal results are not always achieved solely with this treatment. In these cases, resin composite restorations are indicated to achieve the expected results, because they are able to mask discolorations, require minimal or no substrate preparation, and are more affordable than indirect porcelain restorations.²

Masking discolored substrates with direct restorations is a complex procedure, because of the translucency of the resin composites, and requires correct diagnosis, treatment planning, and implementation of a restorative technique that promotes predictable and long-lasting results.³ The multilayering technique using different resin composite translucencies is indicated in performing these procedures, depending on the characteristics of the tooth and the restorative system. When translucent materials are used, the shade of the underlying substrate may pass through the top layers, making the restoration grayish.⁴ This undesirable effect can be minimized by applying an initial layer of an opaque dentin shade to create a masking effect. At the end of the treatment, the restorative procedure should mask the discolored background and make it clinically imperceptible.^{2,5}

The color stability of resin composite restorations is an important parameter for successful clinical results.⁶ Color changes in the resin composite are commonly associated with repairing and replacement of restorations.^{3,7,8} Discoloration of the resin composite is linked to intrinsic and extrinsic factors. The extrinsic changes are related to food pigments and deficiencies in oral hygiene,⁹ whereas the intrinsic factors are related to components and properties of the materials. Primers, inhibitory agents, monomers, water sorption, and degree of conversion of resin composites may influence degradation over time, affecting the clinical appearance of the restoration and decreasing the longevity.¹⁰

The presence of hydrophilic monomers may enhance water absorption and lead to greater discoloration. Conversely, hydrophobic monomers result in

lower water sorption and greater color stability.^{9,11-13} Hydrolytic degradation may also promote variation in opacity and change in the color stability of the resin composite.¹⁰ Long-term studies using a relatively short storage period indicate that the major changes in color occur in the first days after polymerization.^{11,14} The performance of color stability after prolonged aging could simulate the behavior of resin composite restorations closer to the clinical reality, considering that they are expected to remain functional and esthetic for many years.

A previous study found that some resin composite combinations applied by the multilayering technique are able to mask a discolored substrate (C4) immediately.⁵ Long-term masking maintenance was not evaluated. The present study aims at complementing the previously reported initial data with an evaluation of masking ability effectiveness of the multilayering technique, using the same resin composite restorative combinations applied both immediately and 1 year after water aging. The null hypothesis was that aging does not affect the resin composite ability to mask a C4 background.

METHODS AND MATERIALS

This was the second study on masking ability using the same methodology as the first⁵; however, in this study the samples were analyzed after 24 hours of water immersion (T_0) and after 12 months of water aging (T_1). Three groups were formed with different restorative systems. These groups were divided into 18 subgroups ($n=5$), according to the layer-to-layer strategies of the different resin composite shades of each restorative system. The manufacturer, composition, color and translucency, and batch number of each system are presented in Table 1. The group distribution and multilayering technique are shown in Table 2.

Specimen Preparation

One trained and calibrated operator produced the specimens with three cylindrical metallic devices, measuring 11.0 mm in diameter by three different thicknesses: 0.5, 1.0, and 1.5 mm. The total built-up thickness of all the specimens was set at 1.5 mm. The single-layer specimens were prepared with a 1.5-mm-thick matrix, and the resin composite was inserted in a single increment. The two-layer specimens were prepared by first obtaining a 1.0-mm-thick layer from the 1.0-mm matrix. Then, the resin composite disc was placed inside the 1.5-mm-thick matrix, and the remaining 0.5 mm was filled with resin composite and light cured. The three-

Table 1: Composition and Information Regarding the Restorative Systems

Restorative System	Composition	Manufacturer	Shades	Batch Number
IPS Empress Direct	Dimethacrylate, Ba-Al-SiO ₄ glass-oxide silicates, ytterbium fluoride nanoparticles (YbF ₃)	Ivoclar Vivadent	A1 enamel	A1-010040
			A1 dentin	OL-010030
Z350 XT	Bis-GMA, urethane dimethacrylate (UDMA), Triethyleneglycol dimethacrylate (TEGDMA), bisphenol-A dimethacrylate (Bis-EMA), Polyethylene glycol dimethacrylate (PEGDMA), BHT, silicate, zirconia	3M-ESPE, St. Paul, MN, USA	A1E A1B A1D	1415300268
Charisma Diamond	UDMA, TCD-DI-HEA (TCD-urethane), Ba-Al-F glass silicate, YbF ₃ , SiO ₂	Heraeus Kulzer GmbH, Hanau-Hessen, Germany	A1 Universal	A1-010040
			Opaque Light	OL-010030

layer specimens were prepared following the previously described procedures, using 0.5-, 1.0-, and 1.5-mm-thick matrices (Table 3). Each layer was light-cured for 40 seconds using a light-emitting diode (LED; Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) with 1000-mW/cm² irradiance. The upper and lower surfaces of the specimens were covered with polyester strips, and a glass plate with 1 Kgf of static load was applied before polymerization of both sides.⁵

Specimen Storage and Aging

The specimens were stored for one year in deionized water at 37°C, in a transparent plastic container

with a hermetic lid. During this period, the water was changed weekly.¹⁵

Color Measurement

The color measurement of the specimens was performed according to the CIELAB shade scale for the D65 standard light source, on a white background, in a reflection spectrophotometer (SP60, EX Rite/Grand Rapid, MI, USA), at baseline and after aging. The CIELAB color system is a tridimensional color space, based on L*, a*, and b* coordinates.¹⁶ L* refers to lightness, with values ranging from 0 (black) to 100 (white); a* and b* are considered chromatic coordinates: a* for red (+) and green (–), and b* for yellow (+) and blue (–). The mean value of

Table 2: Division of Groups and Subgroups and Description of the Multilayering Technique

Manufacturer	Shades	Layering Technique (n=5)	Layers
IPS Empress Direct (n=5)	A1E – A1 Enamel - E A1D – A1 Dentin - D	1.5 mm E	SL
		1.0 mm E + 0.5 mm D	DL
		0.5 mm E + 1.0 mm D	DL
		1.5 mm D	SL
Charisma Diamond (n=5)	A1U – A1 Universal - E OL – Opaque light - D	1.5 mm E	SL
		1.0 mm E + 0.5 mm D	DL
		0.5 mm E + 1.0 mm D	DL
		1.5 mm D	SL
Filtek Z350 XT (n=5)	A1E – A1 Enamel - E A1B – A1 Body - B A1D – A1 Dentin - D	1.5 mm E	SL
		1.0 mm E + 0.5 mm B	DL
		1.0 mm E + 0.5 mm D	DL
		1.5 mm B	SL
		0.5 mm E + 1.0 mm B	DL
		1.0 mm B + 0.5 mm D	DL
		1.5 mm D	SL
		0.5 mm E + 1.0 mm D	DL
		0.5 mm B + 1.0 mm D	DL
		0.5 mm D + 0.5 mm B + 0.5 mm E	TL

DL, two-layer specimens; SL, single-layer specimens; TL, three-layer specimens.

Table 3: Mean Values of ΔE_{ab} (SD) Before and After Aging for Each Combination^a

Brand	Shade Combination	$\Delta E^*_{ab} T_0$	$\Delta E^*_{ab} T_1$
IPS Empress Direct	1.5 E	7.83 (0.14) ^a	10.79 (0.49) ^a
	1.0 E + 0.5 D	7.05 (0.31) ^b	10.32 (1.11) ^{ab}
	0.5 E + 1.0 D	6.65 (0.10) ^{bc}	7.54 (0.68) ^{cde}
	1.5 D	6.11 (0.20) ^{de}	6.50 (1.23) ^{def}
Charisma Diamond	1.5 E	5.77 (0.24) ^{ef}	9.23 (1.17) ^{abc}
	1.0 E + 0.5 D	5.71 (0.12) ^{ef}	9.29 (0.51) ^{abc}
	0.5 E + 1.0 D	6.08 (0.17) ^{de}	8.34 (0.86) ^{bcd}
	1.5 D	5.58 (0.09) ^f	8.69 (0.43) ^{bc}
Filtek Z350 XT	1.5 E	7.01 (0.25) ^b	10.00 (1.25) ^{ab}
	1.0 E + 0.5 B	6.30 (0.14) ^{cd}	10.13 (0.69) ^{ab}
	1.0 E + 0.5 D	4.99 (0.27) ^g	11.01 (1.10) ^a
	1.5 B	5.73 (0.22) ^{ef}	9.44 (0.56) ^{abc}
	0.5 E + 1.0 B	5.87 (0.12) ^{def}	9.53 (0.46) ^{abc}
	1.0 B + 0.5 D	4.24 (0.12) ^h	8.41 (1.76) ^{bcd}
	1.5 D	2.03 (0.15) ^k	4.56 (0.84) ^f
	0.5 E + 1.0 D	3.36 (0.21) ⁱ	5.35 (0.43) ^f
	0.5 B + 1.0 D	2.84 (0.26) ⁱ	4.81 (0.55) ^f
	0.5 E + 0.5 B + 0.5 D	3.79 (0.06) ⁱ	6.04 (0.24) ^{ef}

^a Different lowercase letters in the same column indicate statistically significant differences. SDs are in parentheses. Bold values indicate adequate masking ability.

L^* , a^* , and b^* was calculated at baseline (T_0) and after one year of water aging (T_1).

Each specimen was analyzed three times, applied over two different backgrounds, with a coupling agent (glycerin) to simulate the oral environment.¹⁷ The different backgrounds simulated two conditions: a severely discolored background and a tooth surface with no discoloration. The first was represented by a 2-mm-thick C4 opaque shade ceramic disc (C4; $L^*=69.18$; $a^*=6.80$; $b^*=23.61$) and the latter by an inherent color background produced with an A1 dentin shade resin composite built up with a 4-mm thickness of each restorative system (Empress Direct: $L^*=82.99$; $a^*=2.46$; $b^*=17.88$ /Charisma: $L^*=81.87$; $a^*=4.31$; $b^*=18.55$ /Z350XT: $L^*=83.72$; $a^*=1.52$; $b^*=16.98$).^{5,18} It was reported in the literature that a 4-mm-thick resin-based composite disc was not affected by the underlying color background and could therefore be considered the inherent color of the resin composite.¹⁷ All readings followed a chairside layering protocol of the resin composite restorative system, with the enamel layer facing upward. The groups that presented only body and dentin shades also followed the same protocol, with the body resin facing upward during color measurement.⁵

The total color difference of the same specimen placed over the discolored (C4) and the inherent backgrounds was calculated according to the CIE-

LAB and CIEDE2000 equations at baseline and after aging (T_0 and T_1).

The CIELAB color difference (ΔE_{ab}) was calculated as follows¹⁹:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where ΔL^* , Δa^* , and Δb^* corresponds to lightness, green-red, and blue-yellow differences of C4 and IC backgrounds, respectively.

The CIEDE2000 color difference (ΔE_{00}) calculation was as follows¹⁹:

$$\Delta E' = [(\Delta L'/K_L S_L)^2 + (\Delta C'/K_C S_C)^2 + (\Delta H'/K_H S_H)^2 + R_T(\Delta C'/K_C S_C)(\Delta H'/K_H S_H)]^{1/2}$$

where $\Delta L'$, $\Delta C'$, and $\Delta H'^0$ refer to lightness, chroma, and hue differences between color measurements, respectively. K_L , K_C , and K_H are the parametric factors for the conditions and illuminating influence, set at 1 in this study.¹⁸ R_T (rotation function) accounts for the interaction of hue and chroma differences in the blue region. S_L , S_C , and S_H are the weighting functions for the color difference adjustment, considering the location variation of L^* , a^* , and b^* coordinates.^{16,19}

Adequate masking ability is achieved when the background color has no influence on the final color of the restoration, resulting in lower ΔE_{ab} and ΔE_{00}

Table 4: Mean Values of ΔE_{00} (SD) Before and After Aging for Each Combination^a

Brand	Shade Combination	$\Delta E^*_{00} T_0$	$\Delta E^*_{00} T_1$
IPS Empress Direct	1.5 E	5.26 (0.13) ^a	7.14 (0.39) ^a
	1.0 E + 0.5 D	4.62 (0.24) ^b	6.62 (0.79) ^{abcd}
	0.5 E + 1.0 D	4.32 (0.04) ^{bc}	4.77 (0.54) ^{efg}
	1.5 D	3.81(0.16) ^{de}	4.06 (0.88) ^{gh}
Charisma Diamond	1.5 E	3.89 (0.18) ^{de}	5.78 (0.80) ^{bcde}
	1.0 E + 0.5 D	3.86 (0.09) ^{de}	5.84 (0.32) ^{abcde}
	0.5 E + 1.0 D	4.08 (0.12) ^{cd}	5.60 (0.54) ^{de}
	1.5 D	3.73 (0.07) ^e	5.43 (0.27) ^{de}
Filtek Z350 XT	1.5 E	5.07(0.20) ^a	7.09 (0.95) ^{ab}
	1.0 E + 0.5 B	4.50 (0.11) ^b	7.16 (0.50) ^a
	1.0 E + 0.5 D	3.31 (0.20) ^f	6.96 (0.51) ^{abc}
	1.5 B	3.85 (0.17) ^{de}	6.61 (0.40) ^{abcd}
	0.5 E + 1.0 B	4.05 (0.09) ^{cd}	6.68 (0.33) ^{abcd}
	1.0 B + 0.5 D	2.70 (0.08) ^g	5.70 (1.04) ^{cde}
	1.5 D	1.27 (0.10) ^j	3.04 (0.57) ^h
	0.5 E + 1.0 D	2.12 (0.12) ^h	3.67 (0.30) ^{gh}
	0.5 B + 1.0 D	1.78 (0.16) ⁱ	3.25 (0.41) ^h
	0.5 E + 0.5 B + 0.5 D	2.44 (0.04) ^g	4.22 (0.17) ^{fgh}

^a Different lowercase letters in the same column indicate statistically significant differences. SDs are in parentheses. Bold values indicate adequate masking ability.

values. In this study, the clinical acceptance threshold of $\Delta E = 3.46$ and $\Delta E = 2.25$ for CIELAB and CIEDE2000, respectively, were considered. Color difference values above these thresholds can be visualized by a layman and cannot be considered acceptable.^{16,20}

Statistical Analysis

The normal distribution was verified by the Kolmogorov-Smirnov test, and the mean values of ΔE_{ab} and ΔE_{00} were submitted to analysis of variance (ANOVA) and Tukey's *post hoc* test. Statistical analysis was performed at $\alpha = 0.05$ using Minitab software (Minitab, Inc, State College, PA, USA).

RESULTS

The mean values of ΔE for each combination taken from the CIELAB and CIEDE2000 color shade systems at T_0 and T_1 are shown in Tables 3 and 4. The lower ΔE value indicates better results. Although the combinations of 1.5 mm D, 1.0 mm D + 0.5 mm E, and 1.0 mm D + 0.5 mm B, all for the Z350XT restorative system, presented clinically acceptable values before aging (T_0), the results did not achieve the threshold parameters associated with clinical acceptability ($\Delta E_{ab} \geq 1.8 \leq 3.46$ and $\Delta E_{00} \geq 1.3 \leq 2.25$) after aging (T_1).¹⁶ All the other combinations did not achieve clinically acceptable

values in any of the periods evaluated (Figures 1 and 2).

Table 5 presents the comparison before and after aging. Most of the combinations presented statistical differences between the two periods (T_0 and T_1). Color analysis according to CIELAB showed that only the 0.5 mm E + 1.0 mm D and + 1.5 mm D combinations of the IPS Empress Direct system over a C4 background did not differ between the periods evaluated. All the other combinations differed statistically between the periods ($p < 0.05$), resulting in an increase in all the values from T_0 to T_1 . CIEDE2000 color analysis exhibited the same behavior.

The ΔE changes were predominantly influenced by b^* coordinate that decreased for Empress Direct and Charisma Diamond and increased for Z350XT. The L^* coordinate remained stable for IPS Empress Direct and Charisma Diamond and decreased for Z350XT. The a^* coordinate had small variations in all the restorative systems.

DISCUSSION

Dark backgrounds are common in teeth that have suffered intrapulpal hemorrhage, trauma, endodontic treatment, discoloration,²¹ and through-and-through class III and IV restorations.⁴ These conditions can be masked with opaque shade resin composites.^{2,22,23} The best results are achieved when

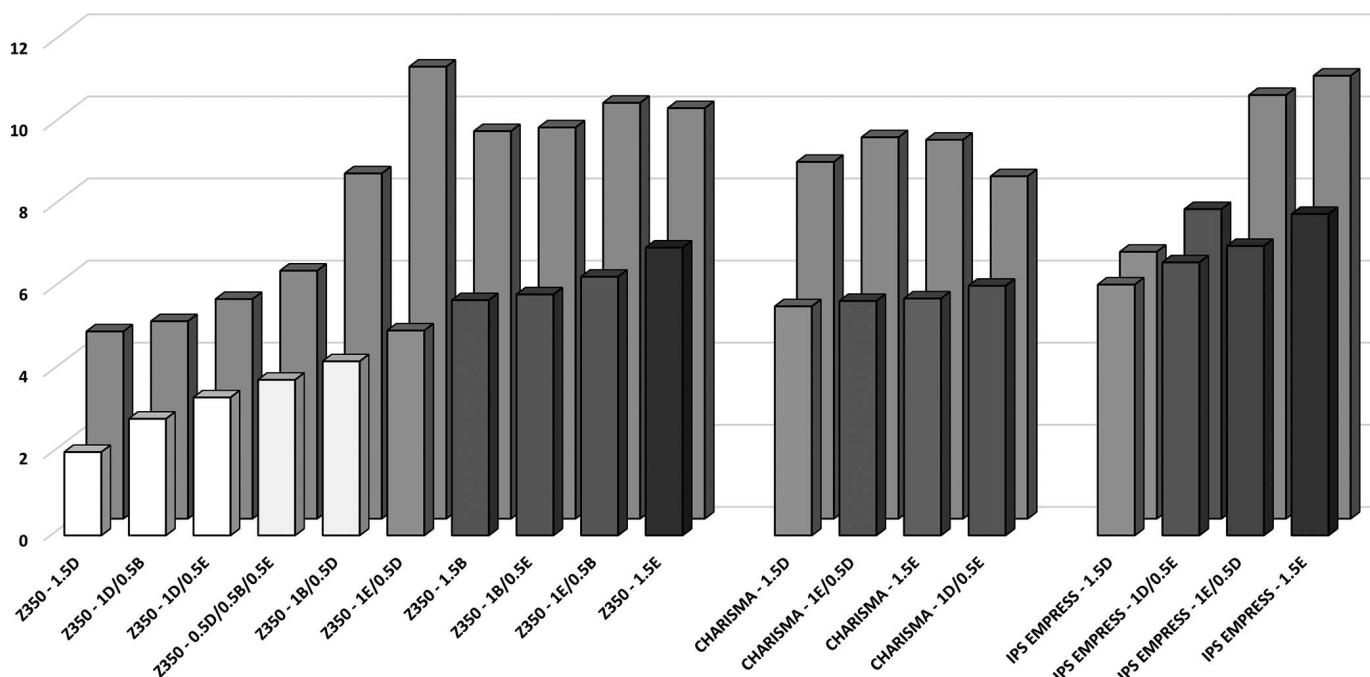


Figure 1. Mean ΔE_{ab} values for different combinations of resin composites before (front) and after (back) aging.

an increased thickness of opaque shade resin composite is used to cover up the discolored substrate.^{2,4,18,23-25} Many studies have evaluated the optical properties of opaque shades alone.^{2,4,18,24} However, the behavior and variations of the layering concept are not often studied.^{5,23,26,27}

Masking ability can be evaluated by the ΔE of the resin composite over black and white backgrounds or by calculating the color difference between the inherent color (IC) of the resin composite and the resin composite placed over a discolored surface.^{2,4} In the present study, a C4 background, representing

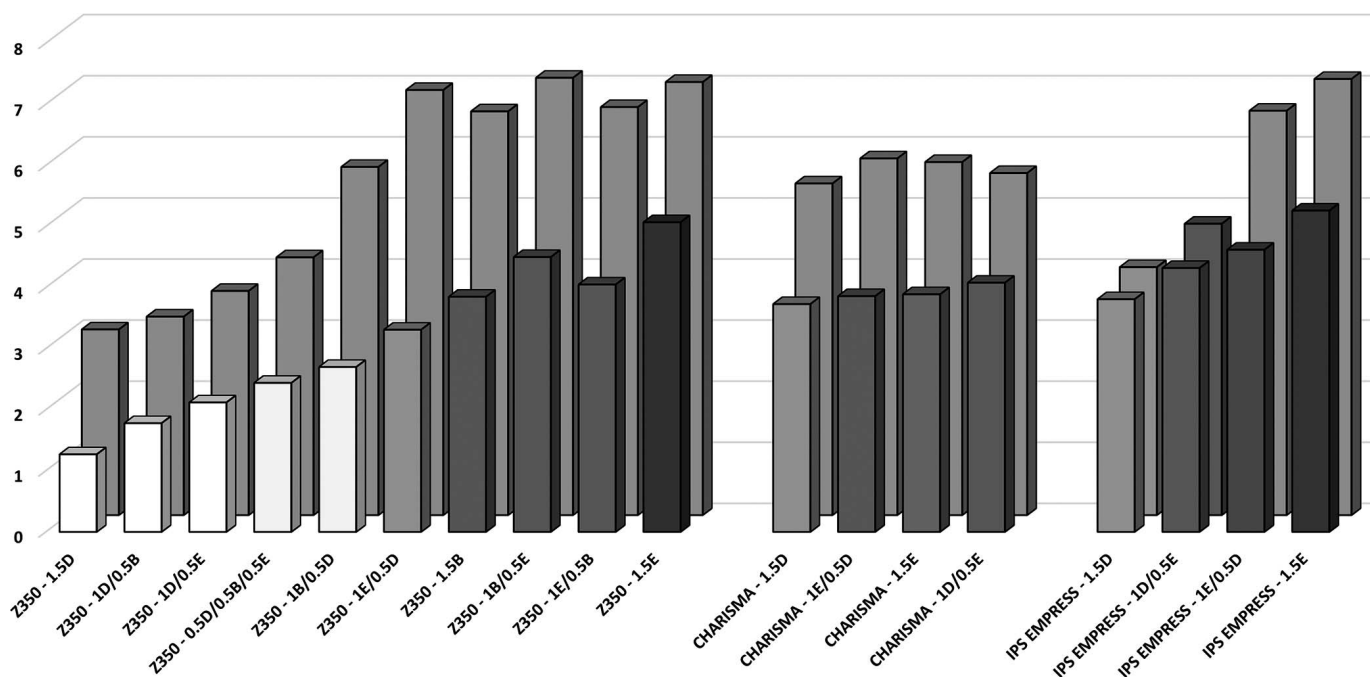


Figure 2. Mean ΔE_{00} values for the different combinations of resin composites before (front) and after (back) aging.

Table 5: Differences in ΔE_{ab} and ΔE_{00} After One Year of Water Aging (Mean Values and SD)							
Brand	Shade Combination	ΔE_{ab}			ΔE_{00}		
		T_0	T_1	p-value	T_0	T_1	p-value
IPS Empress Direct	1.5 E	7.83 (0.14)	10.79 (0.49)	<0.001	5.26 (0.13)	7.14 (0.39)	<0.001
	1.0 E + 0.5 D	7.05 (0.31)	10.32 (1.11)	0.003	4.62 (0.24)	6.62 (0.79)	0.005
	0.5 E + 1.0 D	6.65 (0.10)	7.54 (0.68)	0.053	4.32 (0.04)	4.77 (0.54)	0.150
	1.5 D	6.11 (0.20)	6.50 (1.23)	0.478	3.81 (0.16)	4.06 (0.88)	0.517
Charisma Diamond	1.5 E	5.77 (0.24)	9.23 (1.17)	0.002	3.89 (0.18)	5.78 (0.80)	0.004
	1.0 E + 0.5 D	5.71 (0.12)	9.29 (0.51)	<0.001	3.86 (0.09)	5.84 (0.32)	<0.001
	0.5 E + 1.0 D	6.08 (0.17)	8.34 (0.86)	0.005	4.08 (0.12)	5.60 (0.54)	0.004
	1.5 D	5.58 (0.09)	8.69 (0.43)	<0.001	3.73 (0.07)	5.43 (0.27)	<0.001
Filtek Z350 XT	1.5 E	7.01 (0.25)	10.00 (1.25)	0.004	5.07 (0.20)	7.09 (0.95)	0.005
	1.0 E + 0.5 B	6.30 (0.14)	10.13 (0.69)	<0.001	4.50 (0.11)	7.16 (0.50)	<0.001
	1.0 E + 0.5 D	4.99 (0.27)	11.01 (1.10)	0.001	3.31 (0.20)	6.96 (0.51)	<0.001
	1.5 B	5.73 (0.22)	9.44 (0.56)	<0.001	3.85 (0.17)	6.61 (0.40)	<0.001
	0.5 E + 1.0 B	5.87 (0.12)	9.53 (0.46)	<0.001	4.05 (0.09)	6.68 (0.33)	<0.001
	1.0 B + 0.5 D	4.24 (0.12)	8.41 (1.76)	0.007	2.70 (0.08)	5.70 (1.04)	0.003
	1.5 D	2.03 (0.15)	4.56 (0.84)	0.002	1.27 (0.10)	3.04 (0.57)	0.002
	0.5 E + 1.0 D	3.36 (0.21)	5.35 (0.43)	0.001	2.12 (0.12)	3.67 (0.30)	<0.001
	0.5 B + 1.0 D	2.84 (0.26)	4.81 (0.55)	0.003	1.78 (0.16)	3.25 (0.41)	0.002
	0.5 E + 0.5 B + 0.5 D	3.79 (0.06)	6.04 (0.24)	<0.001	2.44 (0.04)	4.22 (0.17)	<0.001
Bold values indicate $p > 0.05$.							

a substrate with severe discoloration, was used for the evaluation of the masking ability of different A1 shade combinations of three restorative systems. This condition was chosen not only because shade C4 has the darkest and lowest L* value in the Vitapan Classic Shade Guide,² but also because it simulates an extremely challenging situation.^{4,21}

The multilayering technique is a conservative approach for masking discolored backgrounds, because it achieves natural-like results and meets the current esthetic demands.^{22,23,25,27,28} When layering is used, the final color of the restoration is a result of the combined optical properties of all the layers.²⁷ In the present study, it was expected that the combination of A1 shades + C4 background would yield a restoration with the A1 shade or would be within the 50:50% acceptability threshold compared with the IC of the resin composite. When the L, a, and b values of the A1 + C4 combinations are similar to the IC of the restorative systems, the ΔE values are lower. This means that there is less influence of the background on the final color of the specimen + background, and, consequently, greater masking ability.

The CIELAB and CIEDE2000 formulas were used in this study to calculate the masking ability after a prolonged aging period. The CIELAB system calculates the total color difference, using the same

weight for all the coordinates (L*, a*, and b*) in the formula. Conversely, CIEDE2000 uses important adjustments that approximate the analysis to how color change is perceived by the human eye. The formula weighting functions (SC, SH, and SL) adjust the color coordinates relative to chroma, hue, and luminosity, composing the total value for ΔE .^{5,16} Although CIEDE2000 has a more recent formula, both are frequently used and compared.^{5,6,23,29} After one year, the results recorded the same trend for both formulas, but with different absolute values.

The clinically acceptable parameters for CIELAB range between $\Delta E_{ab} \geq 1.8$ and ≤ 3.46 , and those of CIEDE2000 between $\Delta E_{00} \geq 1.3$ and ≤ 2.25 ^{15,16}; that is, in clinical terms, the color variation is only perceived by the human eye when it presents values greater than these. Only a few combinations were able to mask the C4 background at T_0 within the acceptability thresholds, and all had the following common features: the Z350XT restorative system plus a 1.5-mm thickness of dentin shade alone or 1-mm thickness of dentin shade combined with enamel and/or body shades. When the dentin shade thickness decreased to 0.5 mm, the masking ability did not fit in the threshold parameters. This result corroborates study findings that consider thicknesses below 1 mm unable to mask dark substrates^{2,23};

this is especially true for light shades,²³ such as that used in the current study.

In the anterior region, preparation thickness is limited. The creation of space for the layering of two or more shades may lead to additional dental tissue reduction. Therefore, to avoid excessive tooth preparation, resin composite should ideally present low translucency in very thin thicknesses. Conversely, when increments are very thin, resin composites have limited opacification ability.²¹ The favorable results presented by Z350XT may be associated with the translucency pattern of the dentin shade. The Z350XT dentin shade appears to present less translucency than the other systems, whereas the Z350XT body shade seems to behave in the same way as the dentin shade of the other systems.^{5,24} Therefore, the dentin shade acts more like an opaquer than a traditional dentin shade resin composite.

Dental restorative materials must resist wide-ranging adverse conditions, including temperature changes, continuous exposure to moisture, and mechanical stresses.³⁰ Water aging may affect optical properties related to maintaining the masking effect, such as translucency,²⁵ luminosity,^{4,25} and color stability.^{4,22,31,32} Alterations in the translucency parameter can be directly related to changes in masking ability over time. Greater translucency results in increased perception of the background color,²² whereas less translucency may contribute to improving the masking ability.^{4,23}

Considering that masking ability is influenced by the optical properties of the resin composites,²³ and that these features can change over time^{25,32,33} and may influence the final color of the restoration,^{22,23,32} all the restorative systems and combinations were exposed to prolonged water aging regardless of the initial results. Although a positive effect has been previously observed after water exposure,^{6,25,29} this was not the case in the present study; thus, aging was not able to compensate the lack of masking ability.

The research hypothesis was partly rejected, because aging-dependent changes in masking ability depended on the brand of the restorative system and the shade combination. The combinations that were not able to mask the C4 background initially were still unable to mask it after aging, and the combinations that achieved adequate masking ability before aging were not able to mask the C4 background within the acceptability and perceptibility threshold after aging. Hence, it can be inferred

that aging has a negative effect on masking ability. Therefore, when selecting shade combinations and applying the multilayering technique, the instability of the masking ability over time must be taken into consideration.

The IPS Empress Direct restorative system (0.5 mm enamel + 1.0 mm dentin and + 1.5 mm dentin) presented a ΔE value that did not differ between baseline and after aging, both based on CIELAB and CIEDE2000 analysis. Although the masking ability of this combination was clinically unacceptable at both measurements (T_0 and T_1), the resin composite remained stable. This color stability feature is desirable for maintaining both the masking ability over time and the restoration longevity of the esthetic parameters.

Regarding the other combinations, the relation between aging and masking ability may be linked to the composition of the restorative systems, because masking ability^{5,32} and translucency,³³ before and after aging, depend on the shade and the brand of the resin composite system.^{2,32} Water sorption by the triethylene glycol dimethacrylate (TEGDMA) monomer is greater than by the urethane dimethacrylate (UDMA) monomer, and could explain the change in color,³⁴ and, consequently, the loss of masking ability after aging. The presence of TEGDMA in the Z350XT resin composite may explain the change in masking ability presented by the combinations using this material, whereas the absence of TEGDMA would explain the same behavior of the IPS Empress Direct system before and after aging.

Photoinitiators, degree of conversion, and intensity of irradiance may also influence long-term masking ability.³¹ Photoinitiators are formulated as coinitiators, basically by camphorquinone and amines. When the amines are submitted to light or heat, they form a byproduct leading to discoloration that ranges from yellow to brownish-red,³⁵ and that results in intrinsic stains that compromise the esthetic results.³⁶ The yellowish effect is directly proportional to photoinitiator concentration.³⁷ In restorative systems that use camphorquinone, aging is related to an increase in the b^* coordinate of the CIELAB system. The yellowing effect presented by Z350XT after one year of aging, and confirmed by the highest value of b^* , can be attributed to direct oxidation of amines and can negatively influence masking ability.¹⁵

The values of the L^* , a^* , and b^* coordinates suggest that the restorative systems showed discoloration after aging. The L^* coordinate remained

stable in the IPS Empress Direct and Charisma Diamond restorative systems and did not impact the final ΔE . However, the L^* value decreased for Z350XT, showing a tendency to turn gray, which corroborates the findings of previous studies.^{25,30} In all the restorative systems, the ΔE changes were predominantly influenced by the b^* coordinate. The b^* coordinate decreased in IPS Empress Direct and Charisma Diamond but increased in Z350XT. The IPS Empress Direct system showed a tendency toward bluish-red (a^* increased and b^* decreased), corroborated by results found in other studies.^{7,30,38-40} The Charisma Diamond resin showed a tendency toward bluish-green, with a decrease in a^* and b^* values, whereas the coordinate a^* value decreased and b^* value increased in the Z350XT system, tending toward yellowish-green. Although the results demonstrated perceptible color change, the aim of the study was to evaluate masking ability and not color stability after water aging. Nonetheless, these additional data provide relevant clinical information regarding color matching and color stability over time.

The methodology used in the preparation and storage of the specimens in our study followed the same protocol as that of Miotti and others.⁵ A long-term aging protocol of one year was used to promote more predictable clinical conditions, given that aging is normally induced for a period of up to 90 days.^{15,38,41,42} However, there are some limitations in the present study. The aging process was performed only by immersion in deionized water, whereas procedures such as immersion in pigments,⁴² accelerated artificial aging,^{13,15,41} or aging by exposure to ultraviolet light, were not performed.⁴¹ Even so, the aging process used in our study did not compromise the findings and showed additional information concerning the hydrolytic degradation of resin composites.

Despite the constant evolution of resin composites, the masking of discolored substrates continues to pose a great challenge to clinicians, because the restorative systems that allow several layering possibilities do not guarantee clinically acceptable and long-lasting results. Further studies involving the masking of discolored backgrounds using different materials, shades, substrates, periods, and aging techniques are needed to validate this issue.

CONCLUSION

Based on ΔE CIELAB and ΔE CIEDE2000, none of the combinations of the multilayering technique presented adequate masking ability over a severely discolored background after prolonged water aging.

The multilayering technique combining the Z350XT resin composite system with the A1 shade was able to mask a severely darkened substrate at baseline. However, water aging produced a clinically perceptible change, ultimately influencing the masking ability obtained initially. The only system that remained stable after one year of water aging was the IPS Empress Direct in the combinations 0.5 mm enamel + 1.0 mm dentin and + 1.5 mm dentin (A1 shade), despite not presenting adequate masking ability.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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The Combination of CQ-amine and TPO Increases the Polymerization Shrinkage Stress and Does Not Improve the Depth of Cure of Bulk-fill Composites

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Clinical Relevance

The combination of camphorquinone with violet absorption alternative photoinitiators does not improve the depth of cure and, consequently, can increase the polymerization shrinkage stress of bulk-fill composites.

SUMMARY

Objectives: To evaluate the effect of combining camphorquinone (CQ) and diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) on the depth of cure and polymerization shrinkage stress of bulk-fill composites.

Methods and Materials: Experimental bulk-fill composites were produced containing equal molar concentrations of either CQ-amine or

CQ-amine/TPO. The degree of in-depth conversion through each millimeter of a 4-mm-thick bulk-fill increment was evaluated by Fourier transform near-infrared microspectroscopy using a central longitudinal cross section of the increment of each bulk-fill composite (n=3). Light-transmittance of the multi-wave light-emitting diode (LED) emittance used for photoactivation (Bluephase G2, Ivoclar Vivadent) was recorded through every millimeter of each bulk-fill composite using spectrophotometry. The volumetric shrinkage and polymerization shrinkage stress were assessed

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using a mercury dilatometer and the Bioman, respectively. The flexural modulus was also assessed by a three-point bend test as a complementary test. Data were analyzed according to the different experimental designs ($\alpha=0.05$ and $\beta=0.2$).

Results: Up to 1 mm in depth, adding TPO to CQ-based bulk-fill composites increased the degree of conversion, but beyond 1 mm no differences were found. The light-transmittance of either wavelengths emitted from the multi-wave LED (blue or violet) through the bulk-fill composites were only different up to 1 mm in depth, regardless of the photoinitiator system. Adding TPO to CQ-based bulk-fill composites did not affect volumetric shrinkage but did increase the flexural modulus and polymerization shrinkage stress.

Conclusion: Adding TPO to CQ-based bulk-fill composites did not increase the depth of cure. However, it did increase the degree of conversion on the top of the restoration, increasing the polymerization shrinkage stress.

INTRODUCTION

Bulk-fill composites (BFCs) are light-cured, resin-based materials used for the direct restorations of posterior teeth that can be placed in increments up to 4 to 5 mm thick.¹ The higher depth of cure of these composites in comparison to regular resin composites can be related not only to modifications in their monomers and filler compositions but also in the photoinitiator system used. As an example, some manufacturers use some alternative photoinitiators, such as diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (TPO) and benzoyl germanium (Ivocerin) in combination with camphorquinone (CQ), claiming that this combination improves the depth of cure.²⁻⁴

One possible explanation is that these alternative photoinitiators are more reactive than CQ, and they also produce more free radicals capable of initiating the polymerization, thus increasing the degree of conversion.⁵ However, these alternative photoinitiators generally absorb violet light, which has a lower transmittance compared with blue light; thus, they might increase the degree of conversion only on the top of the restoration and might not increase the depth of cure of composites.⁶ While monomers (type and size), fillers (load and size), and pigments might act as competitive absorptive components for both violet and blue light transmission throughout resin-based materials, it remains unclear whether the

photoinitiator system absorption as an isolated factor has a significant effect on BFC light transmission and depth of cure. One question that needs to be asked is whether the increase in degree of conversion on the top would move the refractive index of the polymer formed closer to that of the filler used.^{7,8} This would reduce the mismatch between the resin and filler refractive indexes, improving the transmission of blue light to the deeper portions of the restoration, thus enhancing free-radical formation by the CQ-amine photoinitiator system at the bottom part of the restoration. This would then increase the depth of cure as claimed.

On the other hand, *in vitro* studies have shown that BFC restorations might fail due to internal gap formations or by enamel cracks formed in nearby restoration margins,⁹ possibly correlated to the polymerization shrinkage stress of the composite bulk.¹⁰ Moreover, clinical evidence has shown that BFCs containing CQ-amine combined with TPO had worse clinical performance than BFCs containing only CQ-amine as the photoinitiator system. Interestingly, what is striking in the study of Bayraktar and others¹¹ is that CQ-amine combined with TPO showed more incidence of discontinued anatomic form, marginal discoloration, crevices in which dentin was exposed, and secondary caries in just one year of observation. Therefore, it is possible that the combination of these photoinitiators might not only improve the depth of cure but also play a role in the polymerization shrinkage stress of BFCs with a given resin/filler configuration.

Thus, the aim of this study was to evaluate the effect of combining TPO and CQ on the depth of cure and shrinkage stress of BFCs. The tested hypotheses were that 1) the combination of CQ and TPO will increase the depth of cure of BFCs and that 2) the combination of CQ and TPO will increase the polymerization shrinkage stress of BFCs.

METHODS AND MATERIALS

BFC Formulation

The monomers and filler particles used in the BFC formulation are listed in Table 1. This formulation is based on a BFC formulation patent¹² that has monomers and filler particles with refractive indexes very similar to each other ($n=1.518$ for uncured resin monomer blend; $n=1.549$ for cured resin monomer blend; and $n=1.553$ for the $7.5\ \mu\text{m}$ BaBSiO₂ filler). This reduces light scattering inside the composite, which better identifies the effect of light transmission throughout the BFC.^{7,13,14}

Table 1: Chemical Products Used in Bulk-fill Composite Composition

Material	Chemical	Refractive Index	Concentration (wt%)	Manufacturer
Monomer	Bis-EMA	1.535	16.10	Sigma Aldrich, St Louis, MO, USA.
Monomer	Exothane 24	1.4853	5.75	Esstech Inc, Essington, PA, USA.
Monomer	TEGDMA	1.483	1.15	Sigma Aldrich, St Louis, MO, USA.
Filler particle	16 nm fumed silica	1.535	2	Evonik Industries AG, Essen, Germany.
Filler particle	7.5 μ m BaBSiO ₂	1.553	75	Esstech Inc, Essington, PA, USA.

Abbreviations: BaBSiO₂, barium borosilicate; Bis-EMA, ethoxylated bisphenol A diglycidyl dimethacrylate; TEGDMA, triethylene glycol dimethacrylate.

First, the monomers were blended using a centrifugal mixing device (SpeedMixer, DAC 150.1 FVZ-K, Hauschild Engineering, Hamm, North Rhine-Westphalia, Germany) for 60 seconds at 3000 rpm. To this monomer blend, two different photoinitiator systems were used, as shown in Table 2. The molar ratio concentration of CQ-amine and CQ-amine/TPO were based on previous studies.¹⁵

Then, the fumed silica filler was mixed with the monomer blend for 30 seconds at 3000 rpm followed by the BaBSiO₂ filler for 1 minute at 3500 rpm. Finally, each resin composite was mixed for 1 minute at 3500 rpm under an 80 mmHg vacuum atmosphere.

Light-Curing Unit Characterization

The mean irradiance (mW/cm²) of the multi-wave light-emitting diode (LED; Bluephase G2, Ivoclar Vivadent, Schaan, Liechtenstein) was measured five times using a portable spectrometer-based instrument (CheckMARC, BlueLight Analytics, Halifax, Nova Scotia, Canada) connected to the SpectraSuite software (Spectra Suite 2.0.140, Ocean Optics, Dunedin, FL, USA). The total spectral radiant power output from the LED was divided by the cross-sectional active area (cm²) of the light tip to calculate the average irradiance (mW/cm²). These values were used to calculate the photoactivation time needed to produce a radiant exposure of 20 J/cm². Then, the multi-wave LED was attached to an x-y-z positioning device mounted on an optical bench to standardize the positioning of the light beam in contact with a diffusive surface of a frosted diffuser target (DG20-

1500, Thorlabs, Inc, Newton, NJ, USA) while the resulting image was recorded using a CMOS camera (NEX-F3, Sony Corporation, Tokyo, Japan) with a 50-mm focal length lens. To assess the irradiance distribution at different light emission wavelengths, the CMOS camera beam profiler was used with the addition of bandpass filters (Thorlabs, Inc) placed in front of the camera lens. The bandpass filters were as follows: centered at 400 nm with a 4-nm full width at half maximum (FB400-40) was used to identify the LED chips with spectrum emission peaks at 410 nm and centered at 460 nm with a 10-nm full width at half maximum (FB460-10) was used to identify the LED chips generating emission peaks near 460 nm. As the bandpass filters used in this study have an optical density of approximately 0.2, no neutral density filters were necessary to attenuate the multi-wave LED emission to avoid pixel intensity saturation in the images. To produce calibrated images and data showing the irradiance patterns across the surface of the multi-wave LED, the mean power values, obtained using the CheckMARC were entered into an open source optical analysis software (Fiji, Nature Methods, National Institutes of Health, Bethesda, MD, USA). The scaled numeric data associated with each image was exported into a computer graphic software (Origin Pro, OriginLab Co, Northampton, MA, USA).

Degree of Conversion (DC) in Width and Depth

Each BFC (n=3) was placed in a cylindrical Delrin acetal homopolymer resin mold (12 mm in height, 4 mm in inner diameter) with a Mylar strip manually pressed over the applied material with a glass plate to obtain a flat surface. Afterward, the glass plate was removed and the light curing was performed on the top of the cylinder using the multi-wave LED with 20 J/cm². Then, after 24 hours of dark and dry storage at 37°C, specimens (12 × 4 × 0.5 mm) were obtained by sections from the center of the specimen, perpendicular to the top surface and parallel to the long axis of the cylinder, using an automated water-cooled, low-speed diamond saw (Isomet 1000,

Table 2: Photoinitiator Systems Used in the Bulk-fill Resin Composites

Photoinitiator Systems	Molar ratio CQ:TPO	Concentration (wt%)		
		CQ	EDMAB	TPO
CQ-amine	1:0	0.50	0.58	0
CQ-amine/TPO	1:1	0.25	0.29	0.52

Abbreviations: CQ, camphorquinone; EDMAB, ethyl 4(dimethylamino) benzoate; TPO, diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide.

Buehler Ltd, Lake Bluff, IL, USA)^{6,15,16} with temperature being monitored and recorded during cutting using a IR-Thermal Camera (FLIR Pro, FLIR, Wilsonville, OR, USA). The samples were not submitted to more than 3°C of increase in temperature.

The specimens were fixed onto a glass slab and placed over an automated x-y axis microscope platform. The DC was mapped along the cross section (width 6 mm; depth 4 mm) using a Fourier transform near-infrared (FT-NIR) microscope (Nicolet Continuum, Thermo Scientific, Waltham, MA, USA) coupled to an FT-NIR spectrometer (Nicolet Nexus 6700, Thermo Scientific).^{6,15,17} Every 0.5 mm in depth and width, five infrared spectra were collected from the specimen. The measurements started from 300 µm below the top surface to avoid the area of oxygen inhibition. At each measurement position, the specimens' near-infrared spectra were collected in transmission mode with an aperture of 50 µm, 50 scans/spectrum, and 4 cm⁻¹ of resolution. FT-NIR readings were performed in the transmission mode rather than the reflection mode to evaluate DC in bulk rather than on the surface of the material, which reduces the influence of monomer elution from the surfaces of the section specimens. The spectra of the uncured specimens (n=3) collected with the same settings were used as a reference to measure the peak area ratio corresponding to the aromatic double bonds (4625 cm⁻¹) and vinyl (6165 cm⁻¹) stretching absorptions. The DC (in %) was calculated as follows:

$$DC = \left[1 - \left(\frac{(\text{vinyl peak area}/\text{aromatic peak area})_{\text{pol}}}{(\text{vinyl peak area}/\text{aromatic peak area})_{\text{non-pol}}} \right) \right] \times 100,$$

where pol and non-pol correspond to the area of the methacrylate peak for the polymeric and monomeric states, respectively.

The results were exported to create color-coded maps to describe DC as a function of position in width and depth under the LED emission position using a computer graphic software (Origin Pro, OriginLab Co, Northampton, MA, USA). Each data point registered on the map was the average of three different replications using similar testing conditions. The map scales were set to indicate the maximum DC achieved and a reduction of 10% of the maximum DC in each subsequent color-coded area; a black dashed line was also drawn to represent the limit boundary of 80% of the maximum DC achieved by the BFCs.

Light Transmittance (LT)

The multi-wave LED spectral irradiance (mW/cm²/nm) was obtained with a spectrophotometer (MARC Resin Calibrator, BlueLight Analytics) that has an optical fiber coupled to a cosine corrector sensor of Ø=3.9 mm and a field of view of 180°. The equipment was pre-calibrated using a traceable light source (LS-1-CAL, Ocean Optics) according to the standards of the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). A spectroscopy software (Spectrasuite 2.0, Ocean Optics) was used to obtain the multi-wave LED spectral irradiance over a 380-495 nm wavelength range with a time acquisition of 20 seconds. The graphs obtained were used to calculate the spectral irradiance and the radiant exposure (J/cm²) in the violet range (380-420 nm) and blue range (420-495 nm) by integrating the irradiance versus the wavelength in the spectral irradiance graphs.

LT through each BFC was recorded during the light curing using the same MARC Resin Calibrator spectrophotometer with the same parameters. Samples of each BFC (n=5) were placed in Delrin acetal homopolymer resin molds (Ø=4 mm) of different thicknesses (1 mm, 2 mm, 3 mm, 4 mm, 5 mm, and 6 mm) and positioned on the cosine corrector with Mylar strips covering the top and bottom surfaces of the samples. The spectral radiant power and the irradiance transmitted through each BFC were measured using the spectroscopy software. The spectral irradiance transmitted in the violet and blue ranges were determined for each BFC in different thicknesses. As LT is defined as the percentage of incident light that passes all the way through to the other side of the sample, LT in percent was calculated by subtracting the irradiance obtained at the bottom of each sample from the irradiance of the multi-wave LED in each wavelength range.

Volumetric Shrinkage (VS), Polymerization Shrinkage Stress (PSS), and Flexural Modulus (FM)

VS was measured using a mercury dilatometer (American Dental Association Health Foundation, Gaithersburg, MD, USA).¹⁸ Approximately 0.1 g of each BFC (n=3) was placed on a sandblasted and silanized glass slide (75×25×1 mm). As the glass slide used was 1 mm thick and could attenuate the light that reaches the BFCs during light curing, before starting the test the multi-wave LED was recalibrated to deliver the same radiant exposure (20 J/cm²) through the glass slide. After that, a glass

column was clamped to the glass slide, filled with mercury, and a linear variable differential transducer probe was placed on top of the mercury. VS was monitored for 60 minutes after the photoactivation. The data recorded by the probe was used to calculate the VS using previously measured mass and density values of BFCs.

PSS was measured for 600 seconds using the Bioman.¹⁹ The BFCs were placed between a silanized glass slab and a roughened steel piston. The distance between the glass slab and the steel piston produced a 1-mm-thick composite that corresponded to a configuration factor of 5. The composites were photoactivated through the glass using the multi-wave LED with the same parameters used for the VS test. The load signal in Newton (N) was acquired and then divided by the disk area of the piston to obtain the maximum PSS values (MPa) and the maximum rate of PSS (rPSS, in MPa.s⁻¹).²⁰

As the flexural modulus of the composite is correlated with the magnitude of the polymerization shrinkage stress, the FM was measured using a three-point bend test. Bar-shaped specimens of 10 × 1 × 1 mm (n=10) were made in stainless steel molds. The specimen size of 1-mm thick was chosen according to previous studies to better evaluate mechanical properties.²¹ The bulk increment was light-cured through a Mylar strip using the multi-wave LED with two light irradiations of 20 J/cm², overlapping each other by half of the diameter of the curing light tip. After 24 hours of storage in distilled water at 37°C, the specimens were submitted to a three-point bending test (Instron, Canton, MA, USA) at a crosshead speed of 0.75 mm/min until failure. FM was calculated in gigapascals (GPa) according to the following equation:

$$FM = \frac{L \times D^3}{4 \times w \times h^3 \times d} \times 10^{-3},$$

where L = the maximum load at failure (N), D = distance between the supports, w = the specimen width, h = the specimen height, and d = crosshead displacement.

Statistical Analyses

Data were entered into statistical analysis software (Stata/MP 13, StataCorp, College Station, TX, USA) and checked for normality using the Shapiro-Wilks test and for variance homoscedasticity using the Levene test. Statistical analyses were performed according to the different experimental designs with a level of significance of $\alpha = 0.05$. A power analysis

was conducted to determine the sample size for each experiment to provide a power of at least 0.8 at a significance level of 0.5 ($\beta=0.2$). DC was analyzed using a split-plot analysis of variance (ANOVA) where the independent variables were set as between-subject groups for the experimental BFC (CQ-amine or CQ-amine/TPO) and as within-subject groups for widths (0, 1, 2, 3, and 4 mm) and the depths (0, 1, 2, 3, and 4 mm). LTs in violet and blue range were analyzed independently using a two-way ANOVA where independent variables were set as BFC (CQ-amine or CQ-amine/TPO) and thicknesses (0, 1, 2, 3, 4, 5, and 6 mm). For DC and LT, the Tukey test was applied for multiple comparisons between groups ($\alpha=0.05$). VS, PSS, rPSS, and FM were analyzed using a one-way ANOVA where independent variables were set as BFC (CQ-amine or CQ-amine/TPO).

RESULTS

The multi-wave LED provided a mean irradiance of 1151 ± 50 mW/cm² during the 17 seconds of exposure time, thus producing 20 ± 0.5 J/cm² of radiant exposure. However, 3.4 ± 0.2 J/cm² was within the violet wavelength range (380-420 nm) and 16.6 ± 0.4 J/cm² was within the blue range (420-540 nm) as represented in Figure 1A. Figure 1B,C shows the beam profile of the multi-wave LED according to the violet (380-420 nm) and blue (420-495 nm) wavelength range. The multi-wave LED had an active area of emission of 0.649 cm² and a maximum irradiance of 1386 mW/cm², but the irradiance and the wavelengths were not uniformly distributed across the light tip. In the black dashed circle area referenced to the size of the specimen, the multi-wave LED had an overall irradiance of 1025 mW/cm², where 152 mW/cm² was within the violet wavelength range (380-420 nm) and 873 mW/cm² was within the blue wavelength range (420-495 nm).

Table 3 shows the results of LT into the violet and the blue wavelength ranges for the CQ-amine and CQ-amine/TPO BFCs according to the different thicknesses. For both BFCs, there was an exponential decrease in LT as the specimen's thickness increased, regardless of the wavelength range. However, the CQ-amine BFC had a higher violet light transmittance than the CQ-amine/TPO BFC up to 1 mm in depth, but no differences were found beyond the 1-mm thickness. On the other hand, the CQ-amine/TPO BFC had a higher blue light transmittance than the CQ-amine up to 1 mm in depth, but no differences were found beyond the 1-mm thickness.

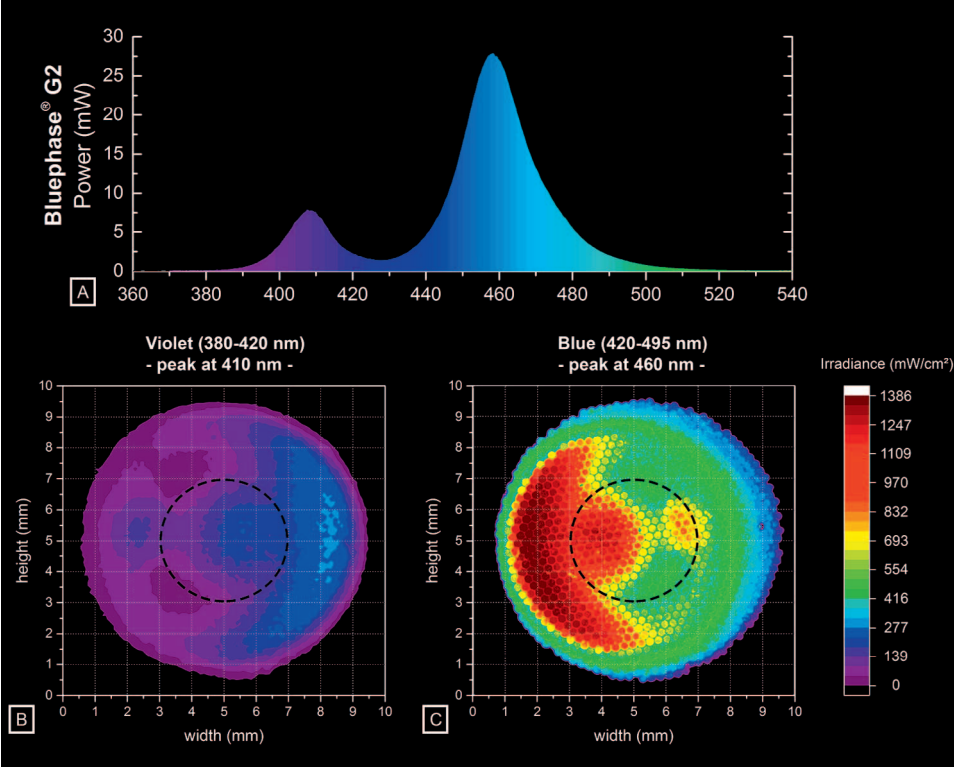


Figure 1. (A) Graph representing the power (mW) versus the wavelength (nm) of the multi-wave LED (Bluephase G2) with a cumulative energy of $20 \pm 0.5 \text{ J/cm}^2$ of radiant exposure. (B) Beam profile of the multi-wave LED with the $400 \pm 40 \text{ nm}$ bandpass filter showing the spectral emission pattern within the violet wavelength range (380-420 nm). The black dashed circle area (---) represents the position, orientation, and area of the specimen onto the multi-wave LED tip. (C) Beam profile of the multi-wave LED with the $460 \pm 10 \text{ nm}$ bandpass filter showing the spectral emission pattern nearly within the blue wavelength range (420-495 nm). The black dashed circle area (---) represents the position, orientation and area of the specimen onto the multi-wave LED tip.

Figure 2A,B show color-coded maps representing, in every 0.5 mm in width and depth, the mean of the degree of conversion (DC, in %) for CQ-amine [A] and CQ-amine/TPO [B] BFCs according to the LED emission position, violet (380-420 nm) and blue

(420-495 nm), in width and in depth. The black dashed line in Figure 2A,B represents the limit boundary where at least 80% of the maximum DC on the top of the restoration was achieved by the BFCs. Figure 2C,D shows areas in yellow where significant

Table 3: Mean $\pm$ SD Irradiance (mW/cm ²) and Light Transmittance (%) Through CQ-amine and CQ-amine/TPO Bulk-fill Composites in Different Thicknesses Within Violet (380-420 nm) and Blue (420-495 nm) Wavelength Ranges ^a					
	Thickness (mm)	CQ-amine		CQ-amine/TPO	
		Irradiance (mW/cm ²)	Transmittance (%)	Irradiance (mW/cm ²)	Transmittance (%)
Violet (380-420 nm)	0	171.0 $\pm$ 5.6	100 Aa	171.0 $\pm$ 5.6	100 Aa
	1	71.3 $\pm$ 5.8	42 Ab	43.8 $\pm$ 1.3	26 Bb
	2	38.3 $\pm$ 0.3	22 Ac	30.4 $\pm$ 1.6	18 Ab
	3	3.6 $\pm$ 2.1	2 Ad	0.0 $\pm$ 0.0	0 Ac
	4	0.0 $\pm$ 0.0	0 Ad	0.0 $\pm$ 0.0	0 Ac
	5	0.0 $\pm$ 0.0	0 Ad	0.0 $\pm$ 0.0	0 Ac
	6	0.0 $\pm$ 0.0	0 Ad	0.0 $\pm$ 0.0	0 Ac
Blue (420-495 nm)	0	980.0 $\pm$ 21.0	100 Aa	980.0 $\pm$ 21.0	100 Aa
	1	398.1 $\pm$ 25.8	41 Bb	449.4 $\pm$ 3.7	46 Ab
	2	257.9 $\pm$ 1.8	26 Ac	237.0 $\pm$ 1.3	24 Ac
	3	172.6 $\pm$ 1.9	18 Ad	170.8 $\pm$ 6.0	17 Ad
	4	123.1 $\pm$ 1.5	13 Ae	120.7 $\pm$ 5.7	12 Ae
	5	77.8 $\pm$ 1.5	8 Af	77.5 $\pm$ 1.9	8 Af
	6	54.0 $\pm$ 2.6	6 Af	55.5 $\pm$ 3.0	6 Af

Abbreviations: CQ, camphorquinone; TPO, diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide.
^a Post hoc Tukey test ($\alpha=0.05$): For each wavelength range (blue or violet), capital letters compare means between photoinitiator systems (CQ-amine and CQ-amine/TPO) and lowercase letters compare means between thicknesses (0, 1, 2, 3, 4, 5, 6 mm).

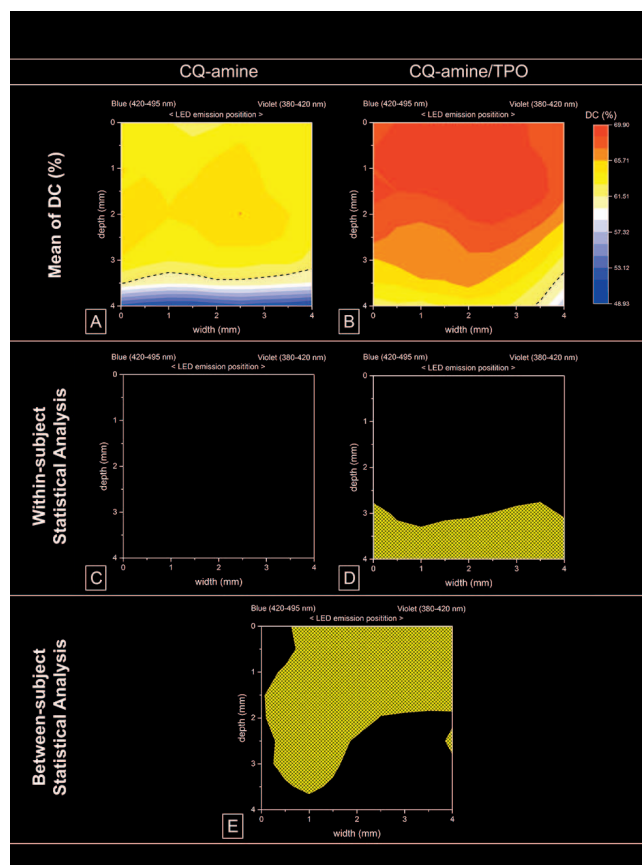


Figure 2. Curing profile of the mean of the degree of conversion (DC, in %) of CQ-amine (A) and CQ-amine/TPO (B) BFCs according to the LED emission position, violet (380-420 nm) and blue (420-495 nm), in width and depth. The black dashed line (---) in the graphs (A) and (B) represents the limit boundary of 80 % of the maximum DC achieved by the BFCs. Panels (C) and (D) show areas in yellow where significant statistical differences were found within subjects (width and depth nested inside each BFC, CQ-amine or CQ-amine/TPO). Panel (E) shows areas in yellow where significant statistical differences were found in the same position (in width and depth) between subjects (CQ-amine and CQ-amine/TPO bulk-fill composites).

statistical differences were found within subjects (width and depth nested inside each BFC, CQ-amine or CQ-amine/TPO). The CQ-amine BFC had a homogeneous DC distribution across the specimen since no statistical differences were found between width and depth regions (degrees of freedom $[df][125]$; $F=1.03$, $p=0.409$). In contrast, the CQ-amine/TPO BFC had a nonhomogeneous DC distribution across the specimen since statistical differences were found between width and depth regions ($df[25]$; $F=1.69$, $p<0.001$), where the areas in yellow showed lower DC than the areas in the top part of the specimen. Figure 2E shows areas where significant statistical differences were found between subjects (for the same width and depth position, differences between CQ-amine and CQ-amine/TPO

BFCs). In the areas presented in yellow, the CQ-amine/TPO BFC had higher DC than the CQ-amine BFC ($df[279]$; $F=30.4$, $p<0.001$)

Table 4 shows the results of VS (%), PSS (MPa), rPSS (MPa.s⁻¹), and FM (GPa) for each BFC. There was no statistical difference between CQ-amine and CQ-amine/TPO BFCs for VS ($df[1]$; $F=1.23$, $p=0.310$). However, the CQ-amine/TPO BFC had a higher PSS ($df[1]$; $F=13.08$, $p=0.011$), rPSS ($df[1]$; $F=35.98$, $p=0.001$), and FM ($df[1]$; $F=6.86$, $p=0.020$) than the CQ-amine BFC.

DISCUSSION

It is believed that LT through a resin-based composite is a result of photoinitiator absorption, surface reflection, light scattering, and refraction caused by differences in the refractive indexes of monomers and filler particles.²² As the same monomers and filler concentrations were used for both experimental BFCs, the present study was designed to determine the effect of two photoinitiator systems in the LT properties of the experimental BFCs. The photoinitiator systems used were CQ-amine, which absorbs light in the blue wavelength range from 420 nm to 495 nm with a peak absorption at 470 nm, and CQ-amine/TPO, which absorbs light in the violet and blue wavelength range from 350 nm to 495 nm with two peak absorptions at 380 and 470 nm.¹⁵ Since several lines of evidence suggest a strong relationship between the spectral output of the light curing unit and the photoinitiator absorption,^{6,22,23} the multi-wave LED used to light cure the BFCs emitted 20 J/cm² of radiant exposure and covered photoinitiator absorption of both CQ-amine and CQ-amine/TPO BFCs.

However, differences were found between the violet and blue wavelength emissions for the multi-wave LED. While 16.6 J/cm² was emitted into the blue spectrum, only 3.4 J/cm² was emitted into the violet spectrum. Nevertheless, despite the differences in the amount of violet and blue light emitted by the multi-wave LED, previous evidence presented thus far supports the idea that the spectral output of the multi-wave LED used was enough to efficiently cure BFCs containing either CQ-amine or CQ-amine associated with alternative photoinitiators.^{6,10,16,26} The present study findings are in agreement with those of previous studies; however these conclusions should be interpreted with further attention since they do not rule out the influence of other factors, such as, the light beam profile received by the specimen.

Table 4: Mean ± SD and One-way ANOVA Results of VS, PSS, rPSS, and FM of CQ-amine and CQ-amine/TPO Bulk-fill Composites			
	CQ-amine	CQ-amine/TPO	One-way ANOVA (α=0.05; β=0.20)
VS (%)	2.20±0.15	2.30±0.17	df(1); F=01.23, p=0.310
PSS (MPa)	2.94±0.07	3.82±0.48	df(1); F=13.08, p=0.011
rPSS (MPa.s ⁻¹)	0.34 ±0.02	0.70±0.12	df(1); F=35.98, p=0.001
FM (GPa)	7.37±0.76	8.46±0.88	df(1); F=06.86, p=0.020
Abbreviations: ANOVA, analysis of variance CQ, camphorquinone; df, degrees of freedom; FM, flexural modulus; PSS, polymerization shrinkage stress; rPSS, maximum rate of polymerization shrinkage stress; TPO, diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide; VS, volumetric shrinkage.			

Therefore, a note of caution is due here since the beam profile results are in accordance with recent studies indicating that the light distribution throughout the multi-wave LED bluephase G2 light tip is significantly influenced by the reflectors, LED chip positions, and the presence of three blue LED chips and only one violet LED chip.^{24,25} Then, the most recognizable finding to emerge from the light curing unit characterization is that the light received on the top surface of the specimens used in this study was not homogeneously distributed, neither in irradiance nor in wavelength. Consequently, the key problem with the nonhomogenous light emission is that it might have important implications for the degree of conversion homogeneity throughout the entire BFC restoration.

But it is somewhat interesting that this is not true for all case scenarios, and the data reported here appear to support the assumption that CQ-amine and CQ-amine/TPO BFCs would have different behaviors regarding the degree of conversion homogeneity when light cured with a nonhomogeneous dual-peak multi-wave LED. To some degree, the CQ-amine BFC had a lower overall DC than the CQ-amine/TPO BFC, but it was homogeneously distributed across the entire specimen. In contrast, the CQ-amine/TPO BFC had a higher overall DC than the CQ-amine BFC, but there were differences between the top and bottom part of the specimen.

It is therefore likely that such connections exist between the position of the violet light beam and the presence of TPO. As shown in Figure 2E, the DC of the CQ-amine/TPO BFC was equal or higher than the DC of the CQ-amine BFC, and this discrepancy is more pronounced in areas where there is an overlap between violet and blue light. But the fact is that the presence of TPO has a significant influence on the DC. TPO is known as a high reactive photoinitiator because it has a higher molar extinction coefficient (≈520 L/mol cm) than CQ (≈28 L/mol cm). This means that TPO absorbs light more efficiently than CQ at their peak wavelength absorptions²⁷ and that

TPO cleavage forms acyl (carbon centered) and phosphonyl (phosphorus centered) free radicals that have higher nucleophilicity and electron resonance than the amino radical (carbon centered) formed by CQ-amine electron donor/hydrogen abstraction reaction. Thus, initiation of polymerization is increased, leading to a higher DC.²⁷

Although these differences in the DC are strictly related to the top part of the restoration due to the TPO wavelength absorption within the violet wavelength range, previous studies have shown that the DC of a polymer-based composite is related to its RI.¹³ Consequently, it is expected that the polymer network with a higher DC formed by the same monomers would have a higher refractive index, closing the mismatch between polymer and filler refractive indexes and improving the LT. As shown in Table 4, the CQ-amine/TPO BFC has a higher blue LT than the CQ-amine BFC, but, contrary to expectations, this difference was found up to 1 mm in thickness, meaning that the improvement in the DC was not enough to improve the blue LT through the BFC. Therefore, the first research hypothesis tested—that the combination of CQ and TPO will increase the depth of cure of BFCs—was rejected.

Nevertheless, a higher DC on the top of the restoration might improve the wear and hydrolytic degradation resistance and perhaps the clinical performance of BFC.²⁸ Therefore, it can thus be suggested that the combination of CQ-amine and TPO would improve chemical and physical properties of BFCs. However, in a more systematic approach this study also identified how CQ-amine and TPO interact with PSS.

Despite no statistical differences being found between the CQ-amine and CQ-amine/TPO BFCs regarding the VS, the combination of CQ and TPO increased the rPSS, PSS, and FM. For CQ-based composites, it is necessary to use an amine together with the CQ to form free radicals efficiently enough to initiate polymerization⁵; however, when CQ and amine are combined with TPO, the reaction could

increase autoacceleration (Trommsdorff-Smith effect).^{29,30}

The major issue is the presence of a tertiary amine (ie, EDMAB) combined with TPO, especially because acyl and phosphonyl radicals formed by TPO cleavage can abstract a hydrogen atom from a tertiary amine, creating one more way to initiate polymerization. This reaction is so fast that it could accelerate the rate of polymerization and the vitrification of the polymer. This means that a very fast reaction does not provide enough time to relax the internal structure, and this shrinkage stress is transferred to the tooth's boundaries.³¹

Furthermore, the stiffness of the material affects the stress produced as a result of polymerization.^{31,32} According to Hook's law, $FM = \text{Stress}/\text{Strain}$, and because the flexural modulus represents the ratio of the elastic stress to the elastic strain, it follows that the higher the stress for a given strain, the higher the FM can be a factor for the higher PSS of CQ-amine/TPO BFC. Therefore, the second research hypothesis tested—that the combination of CQ and TPO will increase polymerization shrinkage stress of BFCs—was accepted.

Recent evidence suggests that the effect of combining CQ-amine and TPO at a molar ratio of 1:1 can improve optical properties without reducing the depth of cure of conventional composites.¹⁵ What is surprising is that perhaps the most serious consequences of combining CQ-amine and TPO are related to PSS and, thus, the clinical performance of BFCs containing CQ-amine and TPO.

Nevertheless, the lack of clinical evidence could lead to a concern regarding the combination of these two photoinitiator systems in BFCs. Bayraktar and others¹¹ showed that a composite containing CQ-amine associated with alternative photoinitiators (TPO and Ivocerin) has a higher incidence of discontinued anatomic form, marginal discoloration, creviced in which dentin is exposed, and secondary caries than a composite containing only CQ-amine in just one year of observation.¹¹ But certainly, this should be observed with more caution because from 50 restorations placed using the composite containing CQ-amine with alternative photoinitiators in this study, only two restorations failed, which means that other factors, such as operator technique, especially during the light curing procedure, might be the cause of these failures. Still, this study seems to have overlooked one important fact that the light curing unit used to cure the CQ-amine with alternative photoinitiators composites was a single

peak blue spectrum monowave LED (Elipar S10, 3M ESPE, St Paul, MN, USA). As the monowave LED used has a single peak emission at 450 nm²⁴ and the BFC used contains TPO and Ivocerin photoinitiators with peak absorption at 370 nm and 418 nm, respectively,⁶ it seems possible that these negative results are due to poor composite light curing.

In addition, Schwendicke and others³³ published a very robust meta-analysis showing the clinical performance of directly placed restorative materials. In that study, 72 randomized controlled trials published between 2005 and 2015 were selected; however, only 8 studies included BFCs. Moreover, only 3 studies were using high-viscosity BFC and none of them included BFCs with alternative photoinitiators. Given that most clinical trials analyzed were short term and showed high risk of bias, future clinical trials should focus on comparative designs and reports using materials with the same composition but different photoinitiator systems.

It is almost certain that the polymerization reaction produces stress that would not be adequately dissipated by the strain caused within the composite, even in BFCs. Therefore, it is probable that the stress is transferred to the bonded interfaces with the tooth structure creating delamination or tooth fracture whenever and wherever the localized stress exceeds the adhesion strength or the strength of the adjacent residual tooth structure. Furthermore, these stresses may increase with time, causing delayed damage to cavity margins. Although many studies have shown *in vitro* evidence of marginal failures using commercially available BFCs, there is abundant room for further progress determining systematically the connections between the combination of photoinitiators and the marginal failures in experimental materials. The findings in this study indicate that CQ-amine/TPO BFCs could have a higher PSS than CQ-amine BFCs. This suggests that a weak link may exist between PSS and the marginal adaptation of BFCs containing CQ-amine associated with TPO.

Nevertheless, new alternatives to improve light curing of BFCs have emerged. Oliveira and others³⁴ showed new composites containing photoinitiators systems that light cured using longer wavelength range (green or red light) than short wavelength range (violet or blue). Apparently, long wavelengths have higher light transmittance than short wavelengths, but further work is required to establish the viability of using these high-efficiency photoinitiators to improve the depth of cure of BFCs. Still, the use of high-reactive photoinitiators in a BFC might

be a drawback since they could increase PSS unless the stress-relief monomer system could compensate for the increasing PSS caused by these photoinitiators. However, recent studies concerning the development of new monomer systems based on thiol-ene polymerization reactions (thio-urethanes)¹⁷ might be a promising alternative to reduce the PSS of BFCs.

CONCLUSION

Within the limitations of this study, the following conclusions can be made: Although the CQ-amine/TPO BFC has a higher DC on the top of the restoration, the combination of CQ-amine and TPO could not increase the blue light transmission to enhance the depth of cure of BFCs. Also, despite the CQ-amine/TPO BFC having some improvement in mechanical properties, such as FM, the CQ-amine/TPO BFC had a higher PSS and rPSS than the CQ-amine BFC.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Bonding Performance of Universal Adhesive Systems Applied in Etch-and-Rinse and Self-Etch Strategies on Natural Dentin Caries

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Clinical Relevance

Universal adhesive systems containing phosphate/carboxylic functional methacrylate derivatives are an interesting alternative when bonding to caries-affected dentin.

SUMMARY

Objective: This study investigated the bonding performance of three universal adhesive systems applied using etch-and-rinse (ER) or self-etch (SE) strategies on natural dentin caries.

Materials and Methods: Sixty human third molars were selected for this study: 30 naturally carious (CAD) and 30 sound (SD) teeth. The dentin surfaces were exposed, and teeth were randomly assigned to each evaluated adhesive system: Scotchbond Universal (SBU), Futurabond U (FBU), and Prime&Bond

Elect (PBE) and an adhesive strategy: ER or SE. The adhesive systems were applied following the manufacturer's instructions, and the teeth were restored using a resin composite (Filtek Supreme Ultra, 3M). After 24 hours (distilled water at 37°C), samples were sectioned and evaluated using microtensile bond strength analysis (μ TBS), micro-Raman spectroscopy to evaluate the degree of conversion within the hybrid layer (DC), and scanning electronic microscopy (SEM) to describe the morphology of the hybrid layer. The μ TBS and DC data were analyzed using three-way analysis of variance and Tukey's test for means

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comparison ($\alpha=0.05$). The SEM images were analyzed qualitatively.

Results: Reduced μ TBS values were observed when comparing CAD with SD, regardless of adhesive system or strategy ($p<0.0001$). SBU showed statistically higher μ TBS for both dentin substrates and strategies ($p<0.0001$). Furthermore, SBU showed greater integrity of the hybrid layer and resin tag formation compared with FBU and PBE. Mean μ TBS values for FBU were higher for SD in the SE mode, whereas higher mean μ TBS values were observed for CAD in the ER mode, both compared with PBE ($p<0.001$).

Conclusion: Bonding performance is reduced on a caries-affected substrate. The ER strategy was not able to improve the bonding performance on natural CAD for universal adhesive systems. Improved bonding performance was obtained when using the Scotchbond Universal system.

INTRODUCTION

In contemporary dentistry, minimally invasive approaches have been consistently developed and reinforced for restorative procedures, with one technique advocating the removal of the outer layer of highly infected, denatured caries-infected dentin.^{1,2} The remaining caries-affected dentin tissue above the pulpal floor is to be preserved because it is a substrate that is suitable for remineralization and could prevent disease progression, reducing the unnecessary exposure of dental pulp tissues.^{1,2}

Several chemical, biological, and physical modifications can occur as a result of the caries process, leading to a clinically distinct situation, which can affect adhesion to caries-affected dentin (CAD).^{3,4} As a result, lower bond strength is found, compared with bonding procedures to sound dentin (SD),⁵⁻⁷ and a poorly formed hybrid layer⁷ can also be observed. However, most of the adhesion studies have focused on investigating SD only, which differs completely from the carious-dentin substrate. Furthermore, some studies are performed in artificially induced lesions,⁸⁻¹¹ which is a less challenging substrate than the one resulting from clinical caries.

In an attempt to simulate the caries process, several artificially induced caries protocols have been described.^{12,13} These methods have a very important advantage in that they provide a standardized substrate, which is difficult to achieve with the natural lesions. However, it is known that no

artificial carious lesion process is able to completely simulate the cascade of events involved in the natural caries process.^{14,15} Additionally, the chemical and histologic characteristics promoted by the natural carious process (water content, blocked dentin tubules due to the deposition of intratubular dentin, among other factors) cannot be simulated.¹⁶⁻¹⁸ Therefore, controversial results are found in the literature regarding different caries induction, bonding, or adhesive strategies.^{4,6,7,9,10,13} This creates difficulty for the clinician when deciding which adhesive system or strategy to use with this sorely modified substrate.

More recently, new “universal” or “multimode” adhesives have been launched in the market.¹⁹ Among the main advantages of these systems is the ability to use either a one-step self-etch approach or phosphoric acid (like an etch-and-rinse system) can be used.²⁰⁻²³ Therefore, the use of universal systems seems favorable due to the broad versatility and facility of use by the clinician.

Unfortunately, there is a lack of dental literature regarding the bonding performance to natural dentin caries, especially when using universal or multimode adhesive systems.¹⁹⁻²³ It is very important to investigate the performance of universal systems when bonding to natural dentin caries, as this substrate represents one of the major reasons for restorative procedures.^{24,25} Unfortunately, the performance of these universal systems has been tested using SD^{22,23,26} or artificially induced caries-affected dentin.^{9,10,13}

The aim of this study was to evaluate the bonding performance of three universal adhesive systems on natural CAD, using both etch-and-rinse and self-etch strategies, compared with sound dentin. The null hypotheses tested were as follows: 1) there is no difference in the bonding performance to natural CAD or SD; 2) the bonding performance is the same for all universal adhesive systems evaluated; and 3) there is no difference between the self-etch and etch-and-rinse strategies on the bonding performance to the tested substrates.

METHODS AND MATERIALS

Tooth Selection and Preparation

Sixty human third molars (30 natural CAD and 30 SD) were obtained with informed consent from donors (20-35 years old) under Local Ethics Committee number 1750969. Teeth were stored at 4°C in 0.5% chloramine T for up to 1 month before use. For carious teeth, the inclusion criteria required teeth

diagnosed with active lesions using tactile, visual, and radiographic analysis, described as a 5 on the International Caries Detection and Assessment System (ICDAS) scale.^{27,28} The samples of carious teeth were prepared by exposing a flat midcoronal carious dentin surface using a slow speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling. One operator was calibrated to achieve appropriate dentin for the lesion walls²⁹ determined by the tactile dentin texture, ensuring that only carious tissue was removed until firm dentin (resistant to hand excavator) was left on the pulpal wall and that the periphery of the lesion contained clean to hard dentin (similar to sound dentin)²⁹ using a hand excavator. The exposed dentin was then finished using 600-grit silicon carbide paper under irrigation with distilled water to standardize the dentin roughness and smear layer thickness. All teeth were thoroughly rinsed with water, followed by the removal of the surrounding enamel using a diamond bur in a high-speed handpiece (#2135, KG Sorensen; São Paulo, SP, Brazil) under water irrigation. All exposed dentin surfaces were further finished using a 600-grit silicon carbide (SiC) abrasive paper for 60 seconds.

Experimental Design

All teeth were assigned to an experimental group based on the type of substrate (CAD or SD) and then three levels for adhesive system: Scotchbond Universal (3M Oral Care, St. Paul, MN, USA, also known as Single Bond Universal in some countries), Futurabond Universal (Voco, Cuxhaven, Germany), and Prime&Bond Elect (Dentsply Caulk, Konstanz, Germany); and two levels for adhesive strategy: etch-and-rinse or self-etch, totaling 12 experimental groups ($n=5$). Detailed information about the composition of adhesive systems, batch numbers, and application modes are described in Table 1.

Bonding and Restorative Procedure

One single calibrated operator performed all adhesive protocols according to each adhesive system and the respective manufacturer's instructions (Table 1). After bonding, the dentin surfaces were restored using a resin composite build-up (Filtek Supreme Ultra, 3M Oral Care). The build-ups were 4-5 mm in height, with three to four increments of resin composite, individually light-cured for 40 seconds using a monowave LED curing unit (Radii Cal, SDI, Bayswater, Victoria, Australia; 1200 mW/cm²). All the restored teeth were stored in distilled water at 37°C for 24 hours prior to preparation.

Specimen Preparation

After 24 hours of storage, teeth were longitudinally sectioned in both the mesio-distal and bucco-lingual directions across the bonded interface in a cutting machine (Buehler, Lake Bluff, IL, USA), resulting in resin-dentin sticks with a 1-mm² cross section. The number of premature failures per tooth during specimen preparation was recorded. The resin-dentin sticks originating from areas immediately above the pulp chamber covered by CAD were selected for testing (selected by dentin color, under 40× magnification). Selected sticks were randomly allocated to three different tests: two sticks for degree of conversion analysis, two for hybrid layer morphology observation, and all other remaining sticks were used for microtensile bond strength testing.

Microtensile Bond Strength (μ TBS)

The cross-sectional area of each stick was confirmed using a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) with a precision of 0.01 mm. Each bonded stick was attached to a jig for microtensile testing using cyanoacrylate resin (Super Bonder Gel, Loctite, São Paulo, Brazil) and subjected to a tensile force using a universal testing machine (Kratos, São Paulo, SP, Brazil) at a crosshead speed of 0.5 mm/min. The failure modes were evaluated under stereomicroscopy at 40× magnification and classified as cohesive, adhesive, or mixed.

In Situ Degree of Conversion Within the Hybrid Layer (DC)

The degree of conversion (on prepared resin-dentin sticks) was analyzed using micro-Raman spectroscopy³⁰ (Horiba Scientific Xplora, Villeneuve d'Ascq, France) with a 785-nm diode laser through a $\times 100/0.9$ NA air objective. The Raman signal was acquired with 600 lines/mm grating, centered between 800 and 1800 cm⁻¹, and the setting parameters were as follows: 100 mW, spatial resolution of 3 μ m, spectral resolution of 5 cm⁻¹, accumulation time of 30 seconds, with five co-additions. Spectra were obtained at the dentin-adhesive interface at three random sites (per stick) within the intertubular-infiltrated dentin. Spectra of uncured adhesives were taken as references. The ratio of double-bonds of uncured and after curing (monomer to polymer) in the adhesive was calculated using the following formula: $DC (\%) = (1 - [R_{\text{cured}}/R_{\text{uncured}}]) \times 100$, where R is the ratio of aliphatic and aromatic peak intensities at 1639 cm⁻¹ and 1609 cm⁻¹ in cured and uncured adhesives, respectively.

Table 1: Adhesive System (Batch Number), Composition, and Application Mode of the Adhesive Systems According to Manufacturer's Instructions

Adhesive System (Manufacturer/Batch Number)	Composition	Application Mode ^a		pH Value ^b
		Self-Etch Strategy	Etch-and-Rinse Strategy	
Scotchbond Universal Adhesive (3M Oral Care, St. Paul, MN, USA; D-82229)	1. Scotchbond Universal Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. 2. Adhesive: 10-methacryloyloxydecyl dihydrogenphosphate monomer, dimethacrylate resins, 2-hydroxyethyl methacrylate, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane.	1. Apply actively the adhesive to the entire surface with a microbrush for 20 s. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely. 3. Light-cure for 10 s at 1200 mW/cm ²	1. Apply etchant for 15 s. 2. Rinse thoroughly. 3. Blot excess water 4. Apply adhesive as for the self-etching mode.	2.8
Futurabond U (Voco, Cuxhaven, Germany; 1346518)	1. Scotchbond Universal Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. 2. Adhesive: 2-hydroxyethyl methacrylate, bisphenolA diglycidyl methacrylate, (poly)methylene dimethacrylate, acidic adhesive monomer, urethane dymethacrylate, catalyst, silica nanoparticles, ethanol.	1. Apply the adhesive to the entire preparation with a microbrush and rub it for 20 s. 2. Directed a gentle stream of air over the liquid for about 5 sec until it no longer moved and the solvent was evaporated completely. 3. Light cured for 10 s at 1200 mW/cm ²	1. Apply etchant for 15 s. 2. Rinse for 10 s. 3. Air dry 2 s. 4. Apply adhesive as for the self-etch mode.	2.3
Prime&Bond Elect (Dentsply Caulk, Konstanz, Germany; 1102221)	1. Scotchbond Universal Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. 2. Adhesive: Mono-, di-and trimethacrylate resins; dipentaerythritol pentaacrylate monophosphate diketone; organic phosphine oxide; stabilizers; cetylamine hydrofluoride; acetone; water.	1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. If necessary, rewet the disposable applicator during treatment. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely. 3. Light-cure for 10 s at 1200 mW/cm ² .	1. Apply etchant for 15 s. 2. Rinse thoroughly with water spray. 3. Air dry. 4. Apply adhesive as for the self-etch mode.	2.5

^a All adhesive systems were applied according to slight modified manufacturers' instructions.^b References 56-58.

Morphologic Analysis of the Hybrid Layer

Specimens were polished using wet SiC paper (grits #1500, 2000, and 2500). After ultrasonic cleaning, specimens were demineralized in HCl (6 N) for 30 seconds and deproteinized in 1% NaOCl for 10 minutes to reveal the hybrid layer. Specimens were dehydrated in ascending grades of ethanol: 25% (20 minutes), 50% (20 minutes), 75% (20 minutes), 95% (30 minutes), and 100% (60 minutes).³¹ Following

preparation, the specimens were mounted and sputter coated with gold-palladium in a vacuum evaporator (SCD 050, Balzers Union, Balzers, Liechtenstein), and the entire surface was examined using a scanning electron microscope (Vega, Tescan, Warrendale, PA, USA). Three photomicrographs of representative surface areas were taken at 5000× magnification.

Table 2: Percentage (%) of Specimens According to Fracture Mode for the Experimental Groups									
Adhesive System	Application Mode	Sound Dentin				Caries Affected-Dentin			
		A/M	CR	CD	PF	A/M	CR	CD	PF
SBU	ER	54 (100)	0 (0)	0 (0)	0 (0)	50 (91)	0 (0)	5 (9)	0 (0)
	SE	50 (98)	0 (0)	0 (0)	1 (2)	48 (92)	0 (0)	4 (8)	0 (0)
FBU	ER	49 (94)	0 (0)	0 (0)	3 (6)	41 (85)	0 (0)	6 (13)	1 (2)
	SE	50 (96)	0 (0)	0 (0)	2 (4)	40 (85)	0 (0)	4 (9)	3 (6)
PBE	ER	52 (95)	0 (0)	0 (0)	3 (5)	38 (83)	0 (0)	5 (11)	3 (6)
	SE	50 (96)	0 (0)	0 (0)	2 (4)	35 (78)	0 (0)	8 (18)	2 (4)
Abbreviations: A/M, adhesive-mixed; CD, cohesive fracture mode in dentin; CR, cohesive fracture mode in resin; ER, etch-and-rinse; PT, premature failures; SE, self-etch.									

Statistical Analysis

The values obtained for specimens from the same experimental unit were averaged for statistical purposes for μ TBS (MPa) and DC (%). Sticks with premature and cohesive failures were not included in the calculation of mean value for the tooth due to their low frequency in the experiment. The Kolmogorov-Smirnov test was used to assess whether the data from these tests followed a normal distribution. Bartlett's test was performed to determine whether the assumption of equal variances was valid (data not shown). After observing normality and equality of the variances, the data from μ TBS (MPa) and DC (%) were subjected to a three-way analysis of variance (type of dentin, adhesive system, and adhesive strategy). Tukey's test was used for pairwise comparisons for all analyses ($\alpha=0.05$).

RESULTS

Microtensile Bond Strength

Approximately 13-15 bonded sticks were obtained per tooth, including the pretest failures. The mean cross-sectional area was $0.94 \pm 0.8 \text{ mm}^2$, and no differences were detected among the experimental groups ($p>0.05$). The failure modes are shown in Table 2. The number of dentin cohesive failures increased in CAD compared with SD.

Means and SDs obtained from μ TBS for all experimental groups are shown in Table 3. The cross-product interaction was statistically signifi-

cant ($p<0.0001$). The higher mean μ TBS values were observed for sound dentin compared with caries-affected dentin, regardless of the adhesive system or strategy ($p<0.0001$).

When Scotchbond Universal (SBU) was applied in the self-etch or etch-and-rinse mode, the highest mean μ TBS values were observed in CAD and SD, which were statistically higher than those obtained for Futurabond U (FBU), and Prime&Bond Elect (PBE) for both strategies in both substrates ($p<0.001$). When FBU was compared with PBE, the former presented higher mean μ TBS values for SD in the self-etch mode and higher mean μ TBS values for CAD in the etch-and-rinse and self-etch mode compared with PBE ($p<0.001$).

When both strategies were compared, the only significant difference was observed for SBU in SD. Higher mean μ TBS values were achieved when SBU was applied using the etch-and-rinse mode compared with the self-etch mode ($p<0.001$).

In Situ Degree of Conversion Within the Hybrid Layer (DC)

The means and SDs obtained from the degree of conversion within the hybrid layer are shown in Table 4. The cross-product interaction was statistically significant ($p<0.001$). Higher mean DC values were observed for SD compared with CAD for both SBU and FBU ($p<0.001$). However, no significant difference was observed for PBE regardless of the type of dentin or adhesive strategy ($p>0.05$).

Table 3: Means and SDs (MPa) Obtained for Microtensile Bond Strength for All Experimental Groups ^a						
Dentin Substrate	SBU		FBU		PBE	
	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse
Sound	46.9 $\pm$ 2.9 b	53.6 $\pm$ 2.9 a	32.0 $\pm$ 2.9 c	32.9 $\pm$ 1.7 c	27.7 $\pm$ 1.4 d	31.2 $\pm$ 1.5 c, d
Caries affected	19.8 $\pm$ 1.6 e	19.2 $\pm$ 1.0 e	13.2 $\pm$ 1.3 f	13.3 $\pm$ 2.6 f	8.6 $\pm$ 1.4 g	9.3 $\pm$ 0.3 g
^a Different letters indicate mean values statistically different (three-way analysis of variance and Tukey's test; $p<0.05$).						

Table 4: Means and SDs (%) Obtained for Degree of Conversion Within the Hybrid Layer for All Experimental Groups ^a						
Dentin Substrate	SBU		FBU		PBE	
	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse
Sound	67.3 ± 3.6 a	68.7 ± 6.9 a	58.7 ± 1.7 b	61.2 ± 6.6 a,b	57.1 ± 2.8 b,c	55.6 ± 2.8c
Caries affected	50.3 ± 4.4 c,d	52.7 ± 4.9 c	50.8 ± 4.3 c,d	44.5 ± 4.6 d	58.1 ± 1.4 b	56.9 ± 2.5 b,c

^a Different letters indicate mean values statistically different (three-way analysis of variance and Tukey's test; p<0.05).

When SBU was applied in either the self-etch or etch-and-rinse modes, the highest mean DC values were observed for SD, which were statistically higher compared with those obtained from FBU (only in the self-etch strategy) and PBE for both strategies and both substrates ($p<0.001$). However, when comparing CAD results, the highest mean DC values were achieved with PBE, which were statistically higher than those obtained from FBU ($p<0.001$). The results from SBU in CAD showed intermediary values.

Morphologic Analysis of the Hybrid Layer

Photomicrographs (5000×) obtained from the morphologic analysis of all resin-dentin interfaces for both SD and CAD by scanning electronic microscopy

(SEM) are shown in Figure 1. For SD, a hybrid layer with more integrity was observed with a greater number and longer resin tags compared with CAD. For all systems, these features were more likely to occur in the etch-and-rinse technique compared with the self-etch technique. CAD images presented hybrid layers with scarce and incomplete tag formation for all adhesive systems.

When comparing the adhesive systems, SBU demonstrated greater frequency of tag formation for both strategies and substrates of FBU and PBE. FBU demonstrated more regular resin tag formation compared with PBE. When evaluating the self-etch mode, resin tag formation was more evident for SD, whereas PBE showed more collapsed collagen fibrils with more porosity signals. FBU demonstrated few

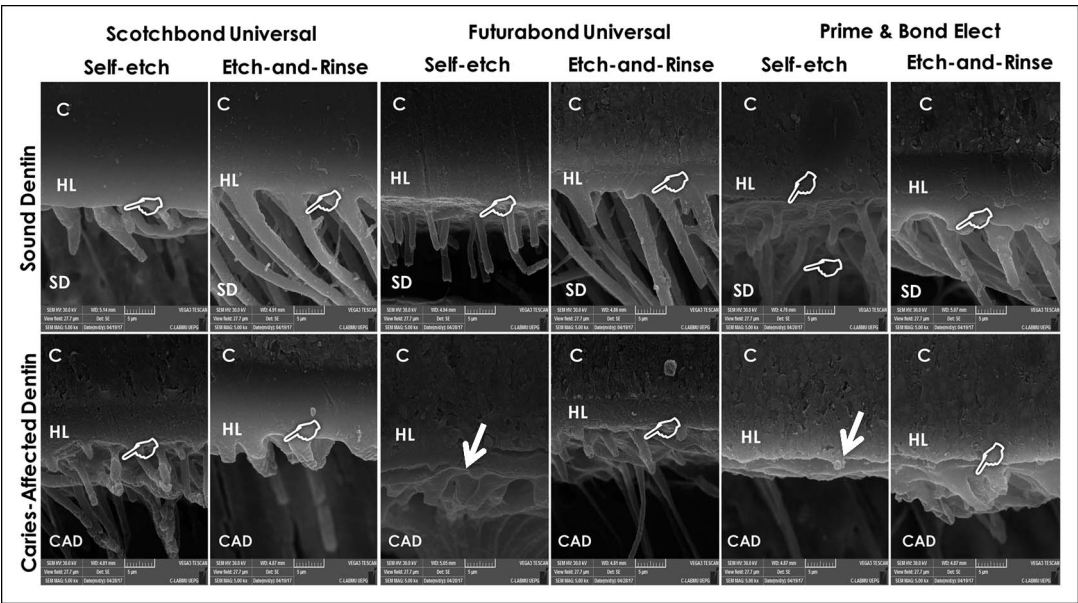


Figure 1. Photomicrographs (magnification ×5000) obtained by scanning electron microscopy of all experimental groups. In the photomicrographs, it is possible to observe the variability of the hybrid layer formation (HL) between the SD and CAD. Note in SD more regularity and integrity of the hybrid layers, showing higher quantity and formation of longer resin tags (hands), mainly when the adhesives systems were applied in etch-and-rinse mode. The HLs promoted on CAD were evidenced by more porous zones, with incomplete resin tag formation. Observe in SBU, the HL showed better resin infiltration promoting HLs with more integrity than in FBU and PBE, regardless of substrate and adhesive strategy. Contrarily, PBE exhibited more disorganized HLs, for SD when applied in the self-etch mode. It is possible to identify confluence of tubules demonstrating poor adhesive penetration and collagen fibril encapsulation, in CAD note the very short and scarce resin tags present for the etch-and-rinse mode, and in the self-etch mode, there is almost total absence of hybridization signals (arrows). For FBU it is possible to observe intermediary hybridization performance, where in CAD, there was slight resin tag formation (arrows) in the self-etch mode and relatively longer tags in the etch-and-rinse mode. Abbreviations: C, composite resin; CAD, caries-affected dentin; HL, hybrid layer; SD, sound dentin.

and short needlelike resin tags with the CAD substrate, although with a higher intensity than PBE. When using the etch-and-rinse mode for the CAD substrate, PBE demonstrated minimal resin tag formation, while resin tags were mostly absent when using the self-etch mode.

DISCUSSION

This is the first study that evaluated the bonding performance of universal adhesive systems on natural dentin caries. Although other studies have evaluated the bonding performance of universal systems on CAD, some in primary dentin,^{9,13} and others in permanent dentin,^{10,32} none of them evaluated natural caries lesions. However, studies involving natural caries lesions have an important disadvantage, which is the difficulty in standardizing the substrate for all evaluated teeth, and this, perhaps, is a potential limitation for studies using this experimental design. Several artificially induced caries protocols have been developed (using acid-gels,³³ pH cycling,³⁴ and microbiological agents³⁵) with the intent of inducing changes to the dental substrate and allowing an easier understanding of the results.¹⁴ However, the chemical caries induction methods have demonstrated controversial results due to the difference between the levels of demineralization and the duration of the demineralization and remineralization cycles not being standardized in pH cyclic protocols.¹⁴ Additionally, the microbiological protocols provide softness, color alterations, and the presence of distinct zones,^{14,35} but there are no standardized protocols for this method³⁶ and the few studies in the literature vary with the type of teeth evaluated, creating a situation where the studies are not comparable with each other.^{14,33,37,38}

The events involved in the natural caries process promote a more dynamic environment. Natural caries is a diffusion-controlled process, involving not only the chemical dissolution of the inorganic material but also the exposure and the degradation of the organic matrix.^{39,40} The different bacterial populations involved in the caries process drop the pH at different times,³⁵ creating a more challenging condition than the laboratory protocols. In addition to demineralization, the exposed organic matrix is degraded by the proteinases from microorganisms of the carious process and the host matrix metalloproteinase activated by bacterial acids, quantitatively and qualitatively affecting the structural substrate.^{15,41} It is likely that these events combine to promote a distinct clinical condition that is,

probably, more difficult to be completely simulated by artificial methods.

The current results showed that the bond strength was reduced when the bonding substrate was caries-affected dentin, regardless of the adhesive system (or technique) used. Thus, the first null hypothesis was rejected. These results were expected because the caries process is associated with the dynamic events of mineral loss and gradual denaturation of collagen fibrils.^{7,42} These events can result in increased porosity in the intertubular dentin,⁷ which can contribute to poor hybridization of the adhesive system for CAD,¹⁸ as evidenced by SEM analysis for all CAD groups. Additionally, the reduced biomechanical properties for CAD compared with SD^{7,42,43} can also be associated with reduced bond strength values for CAD. Unlike the intertubular dentin, the presence of an acid-resistant intratubular mineral deposit¹⁸ can make resin tag formation difficult. According to SEM analysis, this tubule blockage in CAD could have interfered with acid-etching (ER strategy) and monomer penetration (ER and SE strategies) compared with SD.

These factors, such as a decrease in the biomechanical properties of dentin and lack of tag formation due to intratubular deposits, could support the lower bonding performance for CAD. Curiously, even when the current study was performed in natural CAD, the results agreed with other studies that used artificial caries protocols in permanent or primary teeth.^{6,9,13,44,45} It is known that, although the artificial protocols are not able to completely simulate the cascade of events involved in the natural caries process, they do promote isolated alterations in the dentin substrate. According to Marquezan and others,¹⁴ the pH-cycling protocol seems to resemble a natural affected caries dentin layer after caries removal. On the other hand, the microbiological method seems to more adequately simulate a dentin caries lesion with an evident infected layer, simulating a lesion prior to caries removal, although the artificial lesions are softer compared with natural lesions. The selection method of caries induction depends on what the researcher desires to study.¹⁴ Isolated alterations would lead to better understanding the caries process and the potential effect on the bonding performance to a carious substrate. Thus, even when isolated, the alterations would impair the bonding performance compared with SD, consequently explaining the reason why different experimental designs obtained similar findings in bond strength tests.^{6,9,13,44,45}

Our study also evaluated the degree of conversion within the hybrid layer and found reduced bond strength values for CAD, likely due to the lower degree of conversion inside the hybrid layer of two of three adhesive systems. The probable reason for this result is that the higher water content of CAD⁴³ could compromise the photopolymerization of the adhesive systems. Additionally, the phase separation phenomenon could be more prevalent for CAD compared with SD, because hydrophilic monomers tend to penetrate into the wet substrate, whereas hydrophobic monomers penetrate less,^{18,46} which could affect the hybrid layer integrity. Furthermore, the phase separation phenomenon could affect the polymerization and, associated with the higher water content, influence the degree of conversion. However, the degree of conversion for PBE was not affected by the substrate.

PBE was the only evaluated adhesive system with acetone in its composition as a solvent. It is known that acetone demonstrates a higher vapor pressure compared with ethanol and/or water (as used for SBU and FBU), which may facilitate evaporation.⁴⁷ Solvent volatilization can also facilitate the polymerization reaction, because it reduces the distance among monomers, increasing the degree of conversion.⁴⁸ Although adhesive systems containing acetone as a solvent may demonstrate these advantages, a rapid acetone evaporation might not allow enough time for the monomers to infiltrate into dentin. It has been previously reported in the literature that adhesive systems with similar compositions to PBE did not promote uniform adhesive layer thickness across the interface (confocal microscopy), which requires twice the number of applications than recommended by the manufacturer, to obtain an acceptable resin-dentin bond strength.^{26,49} Certainly, this could explain the reduced bond strength values observed for PBE and poor hybrid layer formation, mainly for CAD compared with FBU and SBU. The second null hypothesis was rejected, because different adhesive systems resulted in varied bonding performances.

Among the evaluated universal adhesive systems, SBU exhibited the highest bond strength results, regardless of the dentin substrate and technique. Universal adhesive systems have a composition similar to those of one-step self-etch adhesive systems, and most universal systems also contain specific carboxylate and/or phosphate monomers that can bond ionically to the Ca^{2+} in hydroxyapatite.¹⁹ SBU contains the phosphate acid monomer, 10-MDP (10-methacryloyloxydecyl dihydrogen phos-

phate), which chemically bonds to hydroxyapatite, forming hydrolytically stable calcium salts in the form of "nano-layering" on hydroxyapatite.^{50,51} Additionally, SBU contains a polyalkenoic acid copolymer in its composition, which interacts with apatite substrates following the same adhesion-decalcification reaction.^{52,53} Thus, for SBU, both bonding mechanisms promoted micromechanical retention by diffusion of resin monomers and chemical adhesion. In the CAD substrate, even though it is partially demineralized, there is a reduced content of insoluble minerals in the dentinal tubules, which could be speculated to interact with the mineral content, explaining the morphology of the hybrid layer (SEM) obtained by SBU. FBU and PBE do not contain any of these compounds in their formulations; perhaps, the absence of these functional carboxylic or phosphate derivatives of methacrylate could explain the lower bond strength values compared with SBU. However, further studies are needed to determine whether the presence of carboxylate and/or phosphate monomers are able to maintain the bond strength values when subjected to storage and cycling methods, mainly for CAD.

The third null hypothesis was partially rejected, because the results showed that there is not a significant difference between etch-and-rinse and self-etch approaches for CAD compared with SD, indicating that the application of phosphoric acid is an unnecessary step during the bonding procedures for this substrate. This could be explained by the fact that CAD contains more residual mineral β -tricalcium phosphate¹⁸ (*whitlockite*) in the dentinal tubules, which is less soluble than hydroxyapatite at a pH lower than 5.5⁵⁴ compared with SD. Even though the adhesive strategy for universal systems is still controversial in the literature,^{9,21,22} the bond strength should not be compromised by the strategy used,⁵⁵ because the ionization process resulting from self-etching acidic monomers should promote similar values compared with the etch-and-rinse mode. However, future studies need to be done evaluating which adhesive strategy is better in CAD after long-term exposure to water.

CONCLUSION

The use of the Scotchbond Universal system seems to be an interesting alternative when bonding to CAD, because it can lead to greater bond strengths and adequate hybrid layer formation compared with other adhesive systems tested. The application of phosphoric acid seems to be an unnecessary step

during the bonding procedure to CAD when using universal adhesive systems.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the Ethics Committee of University Ceuma. The approval code for this study is 1.750.969.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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A New Approach for Dental Bleaching Using Violet Light With or Without the Use of Whitening Gel: Study of Bleaching Effectiveness

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Clinical Relevance

Violet light produced clinically significant chromatic changes without the use of bleaching gel and potentiated the bleaching effect obtained with a gel containing 17.5% hydrogen peroxide (HP). However, the use of violet light with a gel containing 35% hydrogen peroxide did not affect the final result.

SUMMARY

The objective of this study was to evaluate the effectiveness of violet light-emitting diodes (LEDs) in dental bleaching treatment when used in conjunction with bleaching gels containing different concentrations of hydrogen peroxide (HP). Here, 90 bovine teeth (n=15) were randomly assigned to the following

groups: GI, placebo without light; GII, 35% HP without light; GIII, 17.5% HP without light; GIV, placebo with violet LED; GV, 35% HP with violet LED; and GVI, 17.5% HP with violet LEDs. Three bleaching sessions of 45 minutes were conducted; 21 cycles involving one minute of irradiation by violet LEDs with 30-second intervals were performed during each session of bleaching (GIV, GV, and GVI). Color changes (ΔE , ΔL , Δa , and Δb) were analyzed using a visible ultraviolet light spectrophotometer 7 days after each bleaching session.

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The mean ΔE , ΔL , Δa , and Δb values were compared between groups by analysis of variance and Tukey tests, with a significance level of 5%. The groups treated with 35% HP had higher ΔE and ΔL and lower Δb values, regardless of whether violet light was used. The group that received only violet LED differed from the control group in terms of ΔE , and the group treated with 17.5% HP and violet LED presented higher ΔE values than the group treated with 17.5% HP only. Thus, violet light did not influence bleaching efficacy when using 35% HP, but when used in conjunction with 17.5% HP, it increased the bleaching efficacy. Moreover, use of the violet LED only also prompted a bleaching effect, although it was less marked.

INTRODUCTION

The search for the perfect smile has led to considerable advances in cosmetic dentistry. A marked proportion of patients seeking to enhance the appearance of their smile choose dental bleaching, which can provide significant esthetic improvements in a relatively short time and at low cost.

In 1989, Haywood and Heymann proposed a home-based dental bleaching technique; it involved application of low-concentration carbamide peroxide-based products, which are used in acetate trays.¹ When properly applied and supervised by a dentist, this technique provides highly satisfactory esthetics.² However, to accelerate the results of bleaching, new techniques and products have been developed, including the adoption of more concentrated products for use in in-office procedures.

Teeth can be lightened due to the permeability of the dental structure to the active compounds present in bleaching gels.³⁻⁵ Reactive oxygen species (ROS) permeate the tooth structure and oxidize the chromophore molecules that are present in dentin and that are the main cause of tooth pigmentation. Oxidation of these substances results in their breakdown, resulting in lighter shades of the treated teeth.^{6,7}

To accelerate and increase the effectiveness of bleaching treatment, products with high concentrations of hydrogen peroxide have been developed and are used in conjunction with different light sources, such as halogen light, light-emitting diodes (LEDs), and laser.^{8,9} The use of light is based on the hypothesis that light projected onto a bleaching product is absorbed and partially converted to heat,

increasing the release of ROS and the efficacy of bleaching. In this way, the light source acts as a catalyst for the degradation of the bleaching product, facilitating its diffusion into the dental structure.^{2,10} However, although this technique is preferred by many practitioners and patients, the use of highly concentrated peroxides may result in exaggerated penetration of peroxide into the pulp tissue, which may further lead to poor health of the pulp.^{11,12}

Moreover, the action of ROS is not limited to oxidation of pigment agents, and reports of excessive concentrations of peroxide in the pulp chamber after application of bleaching gels to the dental enamel are not uncommon.¹¹⁻¹³ This may be associated with mild and transient hypersensitivity, until an inflammatory process that irreversibly affects the pulp develops,¹⁴⁻¹⁸ which can lead to substrate morphologic changes^{19,20} and decreased mitochondrial respiration rate of MDPC-23 odontoblast cells, according to in vitro studies.¹⁹ In vivo studies in human teeth have reported a discreet disturbance in the odontoblastic layer of the pulp tissue of premolars after in-office bleaching treatment,¹⁵ in addition to areas of coagulative necrosis in the lower incisors subjected to bleaching treatment.¹⁸

To ameliorate these side effects, some researchers have recently proposed dental bleaching using violet light (λ : 405-410 nm), with or without concomitant use of bleaching gels.²¹ The wavelength range of violet light coincides with the absorption peak of pigment molecules, interacting selectively with them and causing their breakdown into smaller, colorless components.²¹ However, because this technique has recently been introduced, studies on its actual bleaching effectiveness, as well as its possible side effects are lacking. Several methods have been introduced for analyzing chromatic changes,²²⁻²⁷ but the Commission Internationale de l'Eclairage (International Commission on Illumination [CIE]) system (L^* , a^* , b^*) is one of the most commonly used color analysis methods,^{12,19,28} and surface color analysis is commonly performed in studies analyzing bleaching efficacy.^{10-12,19,26,29}

Thus, there is a need to investigate the possibility of bleaching teeth by exposing them to lower amounts of oxidizing agents. Therefore, this study aimed to evaluate the *in vitro* effectiveness of different bleaching treatments, with or without use of a violet light source. The null hypothesis was that 1) the various bleaching treatments used with or without a violet light source would not vary in their effect on color changes and 2) use of violet light

Table 1: Division of experimental groups according to whitening therapy adopted

Groups	Light	Wavelength	Bleaching Product
No light			
GI	—	—	Placebo
GII			HP 35%
GIII			HP 17.5%
Violet LED			
GIV	Violet LED ^a	405-410 nm	Placebo
GV			HP 35%
GVI			HP 17.5%

^a Fotoclareador Bright Maxx Whitening, MMOptics Ltda.

would not promote a color change when used without a bleaching gel.

METHODS AND MATERIALS

Experimental Design

This factorial randomized study included the following factors: three different bleaching gels (placebo [control], 35% hydrogen peroxide [35% HP], and 17.5% hydrogen peroxide [17.5% HP]); two different light conditions (no light and violet light); and four evaluation times (T0 [before bleaching]; T1 [seven days after the first bleaching session]; T2 [seven days after the second bleaching session]; T3 [seven days after the third bleaching session]).

A total of 90 bovine permanent incisors were randomly divided into six groups (n=15 per group), as shown in Table 1, for use in the study.

Specimen Preparation

Initially, 200 bovine teeth were obtained. All teeth were cleaned with periodontal curettes followed by prophylaxis with pumice and water using a Robinson brush (KG Sorensen Ind. E Com. Ltd, São Paulo, Brazil) at low speeds. To prevent bacterial proliferation, the cleaned teeth were stored in physiologic saline containing 0.1% thymol and kept in a refrigerator at approximately 4°C until the start of the experiment. Dentin and enamel discs measuring 5.7 mm in diameter were obtained with diamond burs used for glass cutting (Dinser Ferramentas Diamantadas Ltd, São Paulo, Brazil). Then, all the discs were subjected to wear by planing of the dentin surface by means of manual rotation on 400 and 600 granulating aluminum oxide polishing discs scales (T469-SF-Noton, Saint-Gobain Abrasives Ltd, São Paulo, Brazil). Discs were planed until they had a thickness of 3.7 mm (1.3 mm of enamel and 2.4 mm of dentin $\pm$ 0.2 mm), measured using digital calipers

(model 500-144B, Mitutoyo Sul América Ltda, São Paulo, Brazil).

Selection and Pigmentation of Samples

After obtaining the discs, they were subjected to an initial reading of the value of L*, using the Visible Ultraviolet Reflection Spectrophotometer apparatus, Model UV-2450 (Shimadzu, Kyoto, Japan). The mean of all samples was determined. We selected 180 dental blocks with L* values closest to the mean value, in accordance with a 5% tolerance level. These 180 enamel/dentin disks were stored in Eppendorf tubes containing a 1-mL infusion of black tea (Matte Leão Tea, The Coca-Cola Company, São Paulo, Brazil) and were monitored for six days, with daily changes of infusion solution. After the pigment treatment, a second color measurement was performed, as described above. Thereafter, 90 pigmented dental blocks were selected for study according to the values of ΔE . After pigmentation with black tea, the specimens were kept in distilled water for six days to remove excess black tea, and the specimens were then subjected to prophylaxis with pumice and water to remove the surface stains.³⁰

The described selection of the specimens was aimed at standardizing the initial color and the intensity of pigmentation of the dental blocks to facilitate standardization of the bleaching capacity of the specimens.

Bleaching Treatment and Irradiation

The 90 stained bovine teeth were randomly assigned to the following groups (n=15 per group): GI, placebo without light; GII, 35% HP without light; GIII, 17.5% HP without light; GIV, placebo with violet LED; GV, 35% HP with violet LED; and GVI, 17.5% HP with violet LED.

The bleaching gel was not applied to the GI and GIV groups, and thus these were considered as

Table 2: Mean values (SD) of ΔE in the different experimental conditions and evaluation times			
Groups	T1	T2	T3
No light			
GI	01.30 (0.56) C a	01.52 (0.42) D a	01.26 (0.50) D a
GII	09.55 (1.44) A c	14.24 (1.77) A b	16.33 (1.55) A a
GIII	05.64 (1.77) B c	09.42 (1.64) B b	11.62 (1.73) B a
Violet light			
GIV	02.66 (1.85) C c	04.25 (1.36) C b	06.48 (1.54) C a
GV	10.24 (1.12) A b	14.10 (1.61) A b	16.10 (1.85) A a
GVI	06.56 (1.66) B c	11.59 (1.21) B b	14.32 (1.47) A a
Means followed by different letters represent significant difference according to statistical analysis (p<0.05). Different lowercase letters indicate statistical difference between columns in the same rows, and uppercase letters indicate statistical difference between rows within the same column. Three-way repeated-measures ANOVA and the Tukey test were performed.			

control groups (Table 1). For the GII and GV groups, manipulation of the 35% HP bleaching gel (Whiteness HP Maxx, FGM Produtos Odontológicos, Santa Catarina, Brazil) was performed according to the manufacturer’s recommendations, by mixing three drops of hydrogen peroxide with a drop of thickening agent. The gel (0.04 mL) was then applied to the enamel surface for 15 minutes. Subsequently, the bleaching gel was removed with cotton balls, and the surface of the enamel was washed with distilled water. Then, the product was reapplied twice more, totaling 45 minutes of contact between the bleaching gel and the dental enamel, in each session. Three such bleaching sessions were conducted, with seven-day intervals between the sessions (Table 1).

For the GIII and GVI groups, bleaching gel containing 17.5% HP was used. For this purpose, a 35% HP bleaching gel (Whiteness HP Maxx, FGM Produtos Odontológicos) was diluted with distilled water. Thus, two drops of the thickener and three drops of distilled water were added to three drops of hydrogen peroxide. The mode of application and the amount of the bleaching gel used were the same as described above (Table 1).

During the bleaching treatment, specimens in the GI, GII, and GIII groups were kept inside a dark chamber to avoid irradiation from any type of light source. The GIV, GV, and GVI groups were irradiated with violet light from an LED light source (Bright Maxx Whitening Light Source, MMOptics Ltda, São Paulo, Brazil), with a wavelength of 405-410 nm. Seven cycles of irradiation from the violet light source were performed at each exchange of the bleaching gel, totaling 21 cycles of irradiation at the end of each bleaching session. Each cycle involved one minute of irradiation followed by 30 seconds of rest (Table 1). At the end of each bleaching session,

the teeth had thus undergone a total of 21 minutes of exposure to light.

Measurements of Color Change

The specimens were subjected to color measurement in a Visible Ultraviolet Reflection spectrophotometer*, Model UV-2450 (Shimadzu). The color measurements were made at the following time points: T0, before the bleaching treatment; T1, 7 days after the first bleaching session; T2, 7 days after the second bleaching session; and T3, 7 days after the third bleaching session.

The CIE system L*, a*, b* calculates the color distance between two points by means of the formula:

ΔE = [(ΔL)² + (Δa)² + (Δb)²]¹/²

Statistical Analysis

The software SigmaPlot version 12.0 (Systat, San Jose, CA, USA) was used for statistical analysis. A power analysis was performed to determine the sample size for each experiment group; a group size of n=15 provided a power of at least 0.826 at a significance level of 0.05. All the data passed the normality test. Three-way repeated-measures analysis of variance (ANOVA) and Tukey’s post hoc tests were used for comparison between groups within time points.

RESULTS

Table 2 shows that all the groups that were treated with bleaching gels or violet light (GII, GIII, GIV, GV, and GVI) presented gradual and continuous color changes at all analysis time points (T1, T2, and

Table 3: Mean values (SD) of ΔL in the different experimental conditions and evaluation times

Groups	T1	T2	T3
No light			
GI	0.31 (0.79) B a	0.47 (0.86) C a	00.15 (0.96) B a
GII	9.33 (3.70) A b	14.61 (4.64) A a	15.07 (4.18) A a
GIII	6.43 (3.62) A b	10.84 (4.15) AB a	11.76 (3.79) A a
Violet light			
GIV	1.20 (2.00) B c	03.08 (2.00) C b	05.03 (2.12) B a
GV	08.51 (2.67) A b	12.11 (2.77) AB a	13.57 (2.39) A a
GVI	04.99 (1.83) AB b	08.74 (2.71) B ab	11.68 (2.73) A a

Means followed by different letters represent significant difference according to statistical analysis ($p < 0.05$). Different lowercase letters indicate statistical difference between columns in the same rows, and uppercase letters indicate statistical difference between rows within the same column. Three-way repeated-measures ANOVA and the Tukey test were performed.

T3). In contrast, specimens from the GI group did not show significant differences throughout the study (Table 2). The comparison of the groups at a given time point showed that, at T1, the groups that received 35% HP treatment were similar (GII and GV), presenting greater chromatic alterations statistically significant than the other groups. Moreover, at T1, groups that received 17.5% HP gel, with or without the use of violet light (GIII and GVI), presented intermediate values, whereas the groups that received placebo gel had the lowest values of ΔE (GI and GIV). At T2, the results were similar to those described for T1; however, the action of violet light was evident in the GIV group, which even with the use of placebo gel presented chromatic alterations larger than those of GI, presenting a statistical difference. At the end of treatment (T3), the groups that received 35% HP gel, with or without light (GII and GV, respectively), and the group that received 17.5% HP with violet light (GVI) had similar values and presented greater chromatic changes than achieved with other treatments. As at T2, the values

of GI and GIV specimens remained statistically different from each other.

Analysis of ΔL (Table 3) shows that GI specimens maintained luminosity throughout the study. The other experimental conditions yielded a gradual and continuous increase of ΔL values over time. At T1, the groups that received bleaching gels had similar values, and the ΔL value of GVI was similar to those of the groups that received the placebo product (GI and GIV). Treatments with more concentrated gels (GII and GV), and treatment with 17.5% HP without violet light (GIII) yielded highest ΔL values at T2. Treatments with placebo gel (GI and GIV) yielded lowest values. At T3, all treatments using bleaching gels (GII, GII, GV, and GVI) yielded similar values and were distinct from the placebo gel (GI) treatment.

Table 4 shows that, in all groups, Δa values were reduced at time T3. Comparisons between groups showed similar performance at all time points, with the highest values obtained with placebo gel treatment, followed by treatment with 17.5% HP gel,

Table 4: Mean Values (SD) of Δa in the different experimental conditions and evaluation times

Groups	T1	T2	T3
No light			
GI	0.66 (0.41) A a	0.75 (0.57) A a	0.47 (0.42) A b
GII	-1.65 (0.88) B a	-0.67 (1.03) B a	-1.79 (1.20) B b
GIII	-0.92 (0.95) C a	-0.74 (1.27) C a	-1.72 (0.85) C b
Violet light			
GIV	0.60 (0.35) A a	0.64 (0.77) A a	-0.23 (0.77) A b
GV	-1.66 (0.85) B a	-1.71 (0.76) B a	-2.64 (0.83) B b
GVI	-0.91 (0.76) C a	-1.01 (0.82) C a	-1.41 (0.86) C b

Means followed by different letters represent significant difference according to statistical analysis ($p < 0.05$). Different lowercase letters indicate statistical difference between columns in the same rows, and uppercase letters indicate statistical difference between rows within the same column. Three-way repeated-measures ANOVA and the Tukey test were performed.

Table 5: Mean values (SD) of Δb in the different experimental conditions and evaluation times			
Groups	T1	T2	T3
No light			
GI	0.67 (0.51) A a	0.28 (0.78) A a	0.08 (0.68) A a
GII	−2.08 (2.40) B a	−4.74 (2.21) BC a	−5.29 (2.99) C b
GIII	−1.02 (1.51) B a	−2.81 (1.34) AB b	−5.91 (1.29) BC c
Violet light			
GIV	0.69 (1.23) A a	−0.91 (2.20) AB a	−2.98 (2.19) AB b
GV	−5.75 (2.94) B a	−7.71 (3.30) C b	−9.43 (2.81) C c
GVI	−3.89 (2.26) B a	−6.46 (2.21) C b	−9.28 (2.06) C c
Means followed by different letters represent significant difference according to statistical analysis (p<0.05). Different lowercase letters indicate statistical difference between columns in the same rows, and uppercase letters indicate statistical difference between rows within the same column. Three-way repeated-measures ANOVA and the Tukey test were performed.			

whereas treatment with 35% HP gel yielded the lowest values. The use of the violet light source did not influence these results.

Table 5 shows that Δb values were reduced for all groups throughout the course of treatment, with the exception of the GI values, which remained constant during the study. In a comparison between the groups, treatment with bleaching gels yielded results, and the lowest values of Δb were seen at T1. At T2, the groups that received bleaching gel along with violet light (GV and GVI), and the group that received 35% HP without violet light (GII) had the lowest, and similar, values. The group that received only violet light demonstrated similar values to those of both groups that received bleaching gels without violet light (GII and GIII) and that of the control group (GI). At T3, all the groups that received bleaching gel treatment (GII, GIII, GV, and GVI) demonstrated similar values, whereas the group that received only violet light irradiation (GIV) had a value similar to that of the control group (GI) and the group that received 17.5% HP gel without violet light (GIII).

DISCUSSION

Despite the numerous protocols published in the literature, bleaching therapies should be considered successful when they are capable of promoting marked chromatic alterations without causing significant side effects.³¹

Although in-office bleaching treatments can provide rapid chromatic changes, they also have significant side effects, as has been shown repeatedly.^{11,12,29,32} In contrast, the at-home techniques, although also effective, present certain disadvantages such as the necessity of the daily use of the tray, slower chromatic alteration, and superficial changes in the dental enamel that could compromise the

adhesion of restorative materials due to saturation of the dental tissues with residual oxygen generated by the degradation of bleaching products.³³

Changing the color of teeth without subjecting them to the typical side effects of therapies based on topical application of peroxides may result in a shift in the esthetic dentistry paradigm and make safer treatments possible. The possibility of using violet light with or without bleaching gels has gained attention among clinicians but has been insufficiently studied.^{21,34} Lago and others reported a clinical case in which this type of light was used for dental bleaching and observed that this technique was effective, changing the tooth color from A3 to A1 in only three bleaching sessions.³⁴ The bleaching sessions were performed using a method similar to that used in the present study, that is, the bleaching sessions lasted 30 minutes, with 20 cycles involving one minute of irradiation with 30-second intervals between the cycles.

The present study evaluated in-office dental bleaching protocol alternatives to identify an approach that could maintain bleaching efficacy while reducing side effects, using a violet LED light source for the partial or total breakdown of chromogenic molecules.²¹ We found that the violet light did not influence the efficacy of the bleaching treatments involving 35% HP, possibly because the high availability of peroxides in the dental tissue masked the action of the violet light. Thus, the two groups that received this gel demonstrated the most significant values of chromatic alteration. Consequently, the first null hypothesis of this study was rejected.

The use of more concentrated bleaching substances favors faster and more intense chromatic changes early during treatment, although the results of the different protocols tended to equalize over longer analysis periods. There have been several reports of

chromatic saturation after three bleaching sessions¹²; however, in the present study that used artificially pigmented teeth, the various treatments still resulted in significant differences even after the third bleaching session. This may be related to pigmentation of the substrate, which allowed values of ΔE higher than 16 to be obtained; this hardly occurs in routine clinical situations, in a naturally stained substrate presents ΔE values between 5 and 8.³⁵

In agreement with our findings, de Oliveira Duque and others compared the performance of a 10% HP-based bleaching gel with that of the traditional 35% HP treatment.³⁶ They observed that the less-concentrated gel was effective as a bleaching therapy; nevertheless, they emphasized that to reach the desired bleaching effect, it was necessary to increase the number of sessions. The authors also observed that the premolar teeth reached saturation of color change after the fifth session, whereas the central incisors saturated in the second session when 35% HP was used. When using an experimental gel based on 10% HP, they observed that the premolars did not reach color saturation even after six sessions of 15-minute applications, whereas in the specimens that simulated the central incisors, saturation was obtained in the fifth bleaching session.

Violet light enhances the efficacy of treatment with a 17.5% HP gel. It is possible that the light emission band of violet light (405-410 nm), which coincides with the absorption peak of pigmented molecules, results in selective interaction with and breakdown of these molecules into smaller and colorless components. These molecules may attain molecular stability when they react with the ROS released by the bleaching product.

The results obtained in the group treated with violet light alone, without any peroxide product, should also be highlighted. This treatment provided a significant chromatic alteration, as compared with that in the control group (G1), at times T2 and T3, negating the second null hypothesis of this study. Although this group presented a less marked bleaching effect as compared with the treatments using gels, the alteration would be clinically perceptible, because they surpassed a value of 3.3, which is considered as visually detectable.³⁷ Therefore, this new technology is promising, and may be feasible for clinical application, enabling bleaching treatment with reduced exposure to peroxides.

When analyzing the coordinates separately, a significant increase in the values of L^* and a

decrease in the values of b^* were observed. On the other hand, the values of a^* varied little and were independent of the treatment used. These findings are in agreement with results reported by Kiomars and others, who emphasized that color analysis in bleaching treatments should consider mainly the former parameters (L^* and b^*).³⁸ It should be noted that L^* identifies an increase in the brightness of the surface of the specimen, whereas b^* represents the color change. In the present study, the decrease in this parameter indicated that there was a decrease in yellowish tones, with more predominant bluish tones in the dental enamel. In this study, in which pigmentation was achieved using black tea, we observed that a^* also showed some differences, indicating that the specimens showed reduced reddish coloration and obtained more greenish tones with treatment.

The color change observed in the group that received only the violet light probably occurred due to the breakdown of pigment molecules by violet light, because pigment remnants were not observed visually in the storage medium, which could have suggested leaching of the pigmentation produced by tea. According to Zanin, the pigment molecules of the dental structure are photoreceptive, and therefore highly reactive to light.²¹ Violet light wavelengths (405-410 nm) coincide with the absorption peak of the pigment molecules present in dental structures, which may result in light activation of the molecules, and their breakdown into smaller, colorless compounds.²¹

Panhoca and others studied the efficacy of violet light in pigmented teeth and obtained results similar to those of the present study.³⁹ However, the authors did not compare the results of light use with the traditional technique involving bleaching gel, emphasizing only that the use of violet light was effective for tooth bleaching. In the present study, it was observed that the isolated light yielded results that were significantly different from those obtained with conventional techniques and increased number of sessions or use in conjunction with bleaching gels were required to obtain a desirable bleaching effect.

The present study had some limitations. First, this was an *in vitro* experimental model using artificially pigmented bovine teeth. Nevertheless, this model has been used extensively in dental bleaching studies, yielding results that were relevant to those observed clinically.⁴⁰ We here used a new technique, seeking to improve the safety of tooth whitening procedures, and needed to standardize conditions and samples, which we could not achieve under

clinical conditions. On the other hand, it is not clear whether our results can be generalized to other pigmentation agents, or whether chromatic stability would remain over longer periods than those analyzed. Because the technology is new, it will be necessary to study the possible adverse effects thereof on adhesion, the mechanical properties of dentin, as well as possible biological effects. Nevertheless, the results of this study revealed that bleaching can be achieved with reduced, or even without, exposure to oxidizing agents, forming the basis for further development of an oxidizing agent-free tooth bleaching process.

CONCLUSION

In this study, we demonstrated that techniques using 35% HP had the greatest bleaching effect, regardless of whether violet light was used. The use of violet light in association with 17.5% HP gel enhanced the bleaching effect. Moreover, violet light alone can also whiten teeth, but to a lesser extent than when used in conjunction with HP-base bleaching gels.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Effect of Conventional and Acid-modified Casein Phosphopeptide-Amorphous Calcium Phosphate Crèmes on Dentin Permeability Before and After Acid Challenge

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S Wipawiwat • S Srikan • S Laojarunghesatchakorn

Clinical Relevance

In vitro application of CPP-ACP-containing crèmes for two weeks has a tendency to reduce dentin hypersensitivity by occluding dentinal tubules.

SUMMARY

This study investigated the effect of conventional and acidified casein phosphopeptide-amorphous calcium phosphate-containing crèmes (CPP-ACP and ACP-ACP) on dentin permeability (DP) before and after acid challenge, using a fluid-flow measuring device and scanning electron microscopic (SEM) examination. Fifty dentin discs were prepared from

intact human third molars. Each tooth was sectioned 3 mm above and below the cemento-enamel junction. The smear layer was removed to expose the dentin and dentinal tubules. For fluid-flow measurement, 20 specimens were used and divided into two groups: (a) CPP-ACP (pH=7) and (b) ACP-ACP (pH=2). The dentin surfaces were treated with the crème for five minutes daily for two weeks, and then an acid challenge was performed using 6% citric acid. Under simulated pulpal pressure, DP was measured at baseline, five minutes, two weeks, and after acid challenge. The remaining specimens were prepared and ex-

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amined using SEM to investigate dentinal tubule occlusion. DP was not significantly different between the two CPP-ACP-containing crèmes at any test period ($p \geq 0.05$). DP after two weeks or acid challenge was significantly lower than that at five minutes ($p < 0.05$). The results were consistent with the SEM micrographs that presented patent dentinal tubules after a five-minute application and partial blockage of dentinal tubules after the two-week application. The dentinal tubules remained partially occluded after acid challenge. Using conventional and acid-modified CPP-ACP-containing crèmes for two weeks decreased DP and partially occluded the dentinal tubules, which resisted acid challenge.

INTRODUCTION

Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic, or chemical stimuli.¹ According to the hydrodynamic theory, dentinal fluid movement within dentinal tubules of exposed dentin is responsible for dentin hypersensitivity.²

Hypersensitive dentin can be conservatively treated by using a desensitizing agent to either block neural transmission or occlude the dentinal tubules.³ In the former, a common desensitizing agent blocking neural transmission is potassium nitrate or potassium chloride.⁴ In the latter, several desensitizing products with the action of occluding dentinal tubules, such as glutaraldehyde, oxalate, fluoride, or resin-based bonding agents, have been introduced.^{5,6} Application of dental lasers may also reduce dentin hypersensitivity by occluding dentinal tubules, coagulating proteins in the dentinal fluid, or altering nerve activity.⁷ Using a laboratory-based dentin disc model measuring dentin permeability (DP) to evaluate the potential of a desensitizing agent or dental laser is a useful screening method.⁸

Casein phosphopeptide-amorphous calcium phosphate-containing crème (CPP-ACP) is commonly used for remineralization of initial carious enamel lesions.⁹ CPP-ACP can also be used as a desensitizing agent in reducing dentin hypersensitivity.¹⁰ The mechanism of CPP-ACP in reducing dentin hypersensitivity is possibly due to its remineralizing action¹¹ and precipitation as hydroxyapatite, which most likely leads to occlusion of the dentinal tubules. Duration of use would be an important factor since a longer application period might

increase the precipitations for desensitization.¹² For this reason, a one- to two-week application period has been suggested.

Improvement of CPP-ACP for desensitizing hypersensitive dentin is possible. A calcium phosphate solution has the highest precipitation rate in acidic conditions at a pH of about 2.¹³ CPP-ACP is likely to be precipitated in the form of a calcium phosphate salt and is effective in reducing dentin hypersensitivity if modified with an acid. It might be assumed that the ability to create a precipitate to occlude dentinal tubules tends to increase when a CPP-ACP-containing crème is modified with an acid.

The desensitizing effect, however, may not be permanent in the oral environment since acidic diets or drinks could dissolve desensitizing products and reopen the dentinal tubules.⁶ Thus, a successful desensitizing agent should withstand acid challenge. Citric acid at 6% has usually been used in laboratory-based studies to test acid resistance of desensitizing agents.⁶

The purpose of this study was to investigate the effect of CPP-ACP-containing crème and an acid-modified CPP-ACP crème (ACPP-ACP) on DP before and after acid challenge in a dentin disc model, using a fluid-flow measuring device and scanning electron microscopic (SEM) examination.

METHODS AND MATERIALS

Fifty intact human mandibular third molars were collected, stored in a 0.12% thymol solution, and used within a week. The roots of the teeth were sectioned 3 mm above and below the cemento-enamel junction using a diamond blade with water coolant (Isomet, Buehler Ltd, Lake Bluff, IL, USA). Pulp tissue remnants were removed with tweezers. The pulpal side was etched with 37% phosphoric acid (ScotchBond Etchant, 3M ESPE, St Paul, MN, USA) for 15 seconds and rinsed with normal saline.

Each sectioned dentin surface was sequentially polished under running water with 600- to 4000-grit silicon carbide papers to remove remnants of enamel and achieve a thin smear layer. The smear layer and smear plugs were removed with 17% EDTA solution (Endo Clean, M-Dent, Bangkok, Thailand) for 60 seconds to simulate exposed dentin with patent dentinal tubules. The dentin surfaces were photographed with a grid of 1 mm² under a polarized light microscope at 40× magnification; a dentin surface area was located and calculated into mm². The

specimens were kept in 0.9% sodium chloride (normal saline) solution before testing.

Dentin Permeability Measurement

Each dentin disc was mounted on a Perspex plate perforated with a 21-gauge needle bonded with cyanoacrylate adhesive. The attached area was subsequently covered with epoxy resin. The pulp chamber of the specimen was filled with normal saline and attached to a DP-measuring device (Mahidol University model, Bangkok, Thailand). DP was measured by detecting fluid by distance within a capillary tube. In the model, simulated pulpal pressure was set at a 14-cm height throughout the experiment.¹² The exposed occlusal dentin surface was saturated with normal saline and covered with a humidified cup to prevent evaporation during fluid flow measurement. To check for any leakage in the system, the device was connected to a root-sectioned tooth with intact coronal structure for 10 minutes, and the absence of fluid movement in the system was confirmed. Fluid movement through untreated dentin was recorded for five minutes and recorded as the baseline DP.

Twenty dentin discs were randomly selected and distributed into two groups (n=10) in each group; the dentin surfaces were treated with either CPP-ACP-containing crèmes (Tooth Mousse, GC Corp, Itabashi-Ku, Tokyo, Japan) or ACP-ACP. The latter was prepared by mixing CPP-ACP with 2 mol/L phosphoric acid solution (pH=1.15), at a ratio of 1:1 by volume and then used immediately after mixing. The paste was applied on a moist dentin surface (agitating with an applicator) for one minute, left undisturbed for four minutes and then removed with a moist cotton swab. Fluid movement was recorded for five minutes as the *5-min DP*.

The specimen was removed from the device and kept in normal saline solution at 37°C for 24 hours. The application procedure was repeated every day for two weeks. Each specimen was reattached to the device, and fluid movement was remeasured for five minutes, as the *2-week DP*. After that, the dentin surfaces were treated with 6% citric acid for five minutes and rinsed with normal saline to remove the acid. Fluid movement was recorded for five minutes after acid challenge, as the *acid-challenge DP*.

DP was calculated from the amount of fluid movement within the capillary tube. Fluid movement, in millimeters, was converted into volume (V) by using the formula, $V(\mu\text{L}) = \pi r^2 \times d$, where r = radius of the capillary tube (150 μm) and d =

distance of fluid movement in μm . DP was then calculated from the formula, $DP (\mu\text{L/s/mm}^2/\text{mm H}_2\text{O}) = V/(T \times A \times P)$, where T = time in seconds, A = area of dentin in mm^2 , and P = simulated pressure in $\text{mm H}_2\text{O}$. Compared with the baseline value, the percentage change in DP was calculated from the formula, $DP_P - DP_B/DP_B \times 100$ (DP_P =DP at a measuring period; DP_B =P at baseline). For percentage change in DP, a negative value (–) indicated a decreased DP while a positive value (+) meant an increased DP.

Scanning Electron Microscopic Examination

Thirty dentin discs were prepared as previously described. Eighteen discs were randomly divided into two groups according to the two CPP-ACP crèmes, nine of each. The specimens in each group were further divided into three subgroups following the three observation periods: five-minute and two-week applications and after acid challenge (n=3 per subgroup). Three untreated discs were used as the control. In addition, CPP-ACP-containing crèmes were diluted with distilled water at a ratio of 1:1 by volume and applied on nine dentin discs, three for each observation period, to observe the dilution effect of CPP-ACP on precipitation. The dentin surfaces were prepared and examined using SEM at a magnification of 2000 $\times$.

The dentin discs were dried, gold sputter-coated, and observed under SEM (JEOL JSM-6610LV, JEOL Inc, Peabody, MA, USA) at a magnification of 2000 $\times$. Each dentin disc was initially observed at low magnification, and then a representative area of treated dentin was chosen and recorded at the desired magnification.

Statistical Analysis

DP in percentage change from the baseline value was analyzed among the three periods in each paste using the Friedman test and Wilcoxon signed rank test. DP of the two crèmes were statistically compared at each period using the Mann-Whitney U-test. The significance level was set at 0.05.

RESULTS

Dentin Permeability

DP (%) changes from baseline values are presented in Figure 1. After application of CPP-ACP for five minutes, two weeks, and after acid challenge, the DPs were –0.45%, –64.99%, and –62.84%, respectively. After application of ACP-ACP at the three

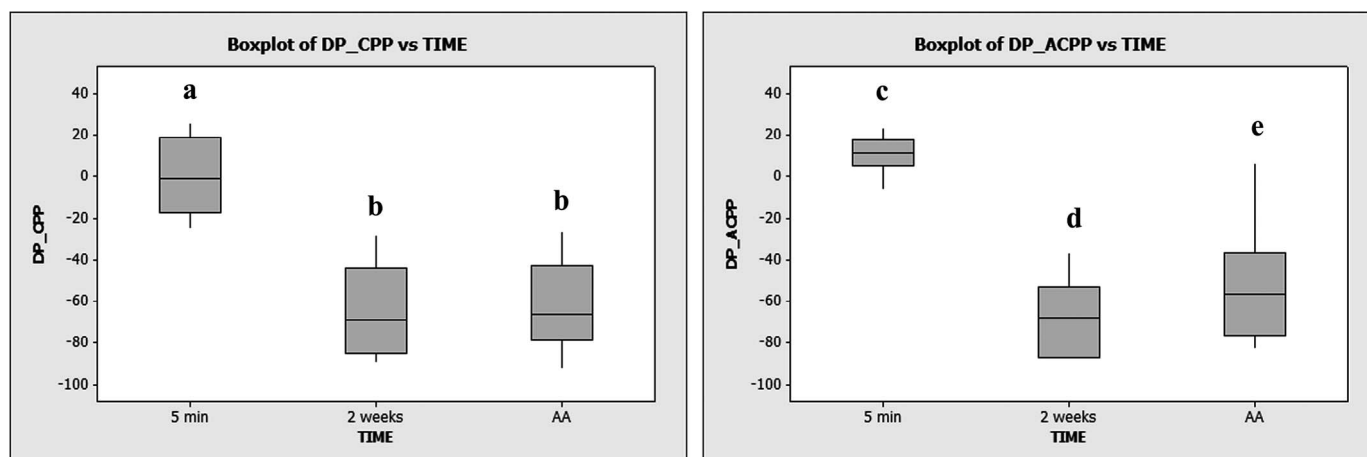


Figure 1. Box plot representing percentage change of dentin permeability (DP) from baseline after application of CPP-ACP and ACP-ACP at five minutes, two weeks, and after acid challenge (AA). The different labels indicate a significant difference among DP at the observation periods within a group. No significant difference was found between DP of CPP-ACP and ACP-ACP at any time period.

periods, the DPs were +10.91%, -68.39%, and -52.52%, respectively.

Comparing among periods, the DP of CPP-ACP was significantly different ($p < 0.01$). DP at either two weeks or after acid challenge was significantly lower than that at five minutes ($p = 0.018$). However, DP at two weeks and after acid challenge was not significantly different ($p \geq 0.05$).

Comparing among the periods, DP of ACP-ACP was significantly different ($p < 0.01$). DP at either two weeks or after acid challenge was significantly lower than that at five minutes ($p = 0.028$). In addition, DP at two weeks was significantly lower than that after acid challenge ($p = 0.018$).

At five minutes, two weeks, or after acid challenge, DP of CPP-ACP application was not significantly different from that of ACP-ACP ($p \geq 0.05$).

Scanning Electron Micrographs

From the SEM micrographs, it was seen that the smear layer and plugs were completely removed from the dentin surfaces treated with 17% EDTA (control), with patent dentinal tubules (Figure 2A). After 5-minutes' application with CPP-ACP, fine-grained particles were partially observed on the dentin surface, with most tubular orifices remaining patent (Figure 2B). After the two-week application, the tubule orifices were partially blocked with deposits (Figure 2C). After the acid challenge with 6% citric acid, the dentinal tubules remained partially occluded, but to a lesser degree (Figure 2D).

Using ACP-ACP for five minutes, 2-4- μ m particles were partially observed on the dentin surfaces,

with patent dentinal tubules (Figure 2E). After the two-week application with ACP-ACP, the dentinal tubules were partially occluded with precipitates (Figure 2F). After acid challenge, the dentinal tubules remained partially blocked, but patent tubules were also observed in some areas (Figure 2G).

Diluted CPP-ACP-containing crèmes did not induce any precipitation at any of the observation stages, and all dentinal tubules remained patent (Figure 2H,I).

DISCUSSION

CPP-ACP-containing crèmes reduced DP and produced a partial blockage of dentinal tubules after two weeks' of daily application. The main precipitate from CPP-ACP is dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$).¹³ However, the incomplete ability to decrease DP and occlude the dentinal tubules may be explained by a slow release of calcium and phosphate ions to form precipitates when CPP-ACP is neutral (pH 7).¹³ Theoretically, dilution of CPP-ACP may enhance the release of ions and possibly improve the precipitation rate, but the release at neutral pH is much lower than under the acidic condition.¹⁴ However, the effect on improving precipitation by diluting the CPP-ACP was not found in this study, wherein the dentinal tubules remained patent even after two weeks. Therefore, DP was not tested in the diluted CPP-ACP group in the current study.

CPP-ACP-containing crèmes should be consecutively applied for a period of time to establish and maintain optimal desensitizing effect.¹² For a five-

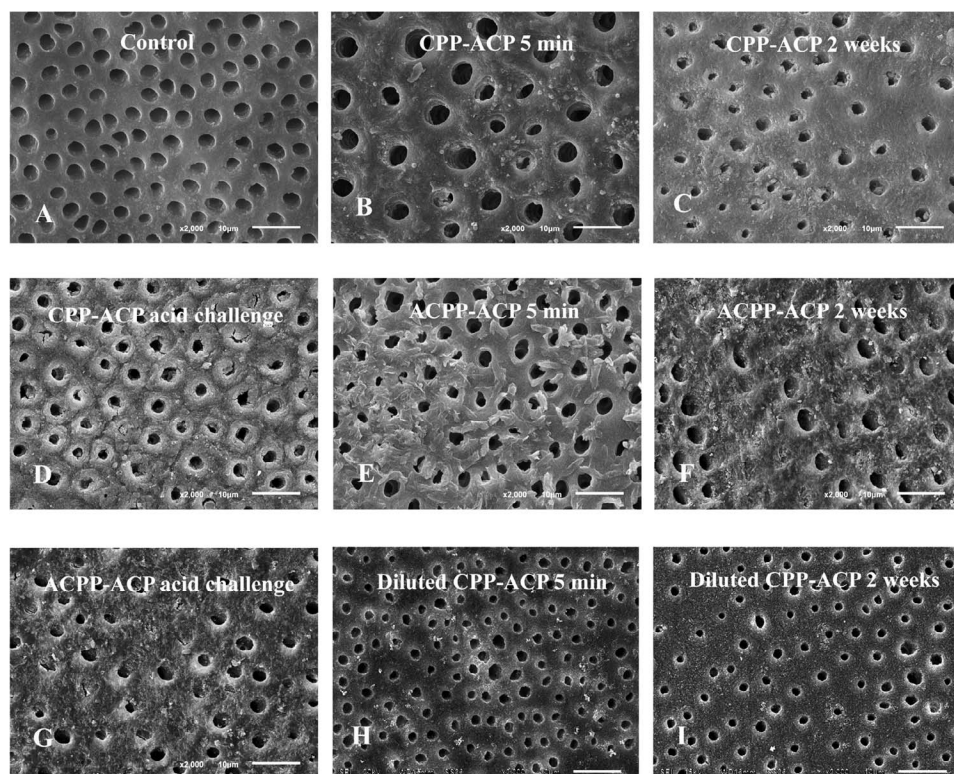


Figure 2. Representative SEM micrographs ($\times 2000$ magnification) of dentin surfaces treated with CPP or ACP at three different intervals. (A) Control; dentin treated with 17% EDTA presented patent dentinal tubules without smear layer or plugs. (B-D) CPP-ACP-containing crèmes at five minutes, two weeks, and after acid challenge with 6% citric acid. At five minutes, fine-grained particles were observed partially covering the dentin surface, with most tubular orifices remaining patent (B). At two weeks, the dentinal tubules were partially blocked with deposits (C). After acid challenge, the tubular orifices remained partially occluded, but to a lesser degree (D). (E-G) ACP-ACP at five minutes, two weeks, and after acid challenge. At five minutes, particles, approximately $2-4 \mu\text{m}$ in size, were observed, with patent dentinal tubules (E). At two weeks, the dentinal tubules were partially occluded with precipitates (F). After acid challenge, the dentinal tubules remained partially occluded, but patent tubules were observed in some areas (G). (H-I) CPP-ACP-containing crèmes diluted with distilled water applied for five minutes or two weeks. All dentinal tubules remained patent without any precipitation that was similar to the observation after acid challenge.

minute application, CPP-ACP was not effective in reducing DP or occluding the dentinal tubules; the majority of tubule orifices remained patent. This finding supports the clinical result by Kowalczyk and colleagues,¹⁵ who reported that the therapeutic effect of CPP-ACP in relieving dentin hypersensitivity was insufficient when used over a short period. From our study, CPP-ACP should be used for at least two weeks to decrease DP and achieve a desensitizing effect. In addition, acidic foods or drinks should be avoided during the period because a lesser degree of tubular occlusion was observed after acid challenge.¹⁶

Theoretically, ACP-ACP had a higher tendency to induce precipitation occluding dentinal tubules and decrease DP than did CPP-ACP. Microparticles were observed on the dentin surface after five-minute application. Immediate precipitation of ACP-ACP is probably due to a rapid precipitation rate in the low pH environment as a calcium phosphate solution has been shown to have the highest precipitation rate at a pH of about 2.¹³ ACP-ACP was a diluted form of CPP-ACP that could increase the release of calcium and phosphate ions,¹⁴ but the enhancing effect on precipitation

was not found when CPP-ACP was diluted with distilled water. Thus, DP at five minutes was not reduced due to the limited effect of immediate precipitation. At two weeks, occlusion of the dentinal tubules when using ACP-ACP tended to be more complete than with CPP-ACP. However, the results of DP were not different between the two pastes.

Apart from the precipitation, ACP-ACP might interact with the dentin surface and dentinal tubules because of its slightly acidic nature. In evaluating the SEM image, however, we noted that the surface seemed to have become rougher and the tubules wider. This etching effect of ACP-ACP should have increased DP. However, the effect of precipitates was likely to be stronger, so the DP was decreased after application of ACP-ACP.

In this study, 6% citric acid was used to determine acid resistance of the precipitates. The SEM images showed that the precipitates occluding the dentinal tubules of the ACP-ACP paste were less likely to be affected by the acid challenge than was CPP-ACP. This might be explained by the larger particles and the possibly faster rate of precipitate formation in the ACP-ACP. After the two-week application,

ACPP-ACP might induce a thicker precipitate layer, which is likely to resist the acidic conditions. However, DP after acid challenge was not different between CPP-ACP and ACPP-ACP.

In the ACPP-ACP group after acid challenge, the standard deviation of DP seemed to be higher than in the other groups. This may be explained by their capacity to resist the acid of the precipitation layer, which varied slightly among the specimens. As seen in the SEM image, the dentinal tubules in some areas were still protected while those in other areas were patent after acid challenge.

CONCLUSION

A two-week application of CPP-ACP-containing crèmes or ACPP-ACP decreased DP and induced dentinal tubule occlusion that resisted an acidic challenge. However, the efficiency in reducing DP of the CPP-ACP crèmes in a laboratory study may differ from the clinical situation with the presence of saliva, biofilm, or repeated acidic challenges. It is necessary to evaluate the clinical efficacy of the CPP-ACP-containing crèmes to confirm these laboratory results.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of Mahidol University. The protocol was approved by the Faculty of Dentistry and Faculty of Pharmacy Mahidol University Institution Regional Board (2015/032.1111).

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Optical Stability of High-translucency Resin-based Composites

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Clinical Relevance

Resin-based composites are able to retain a high-translucency character upon storage.

SUMMARY

This study investigated the stability of the optical properties of high-translucent shades of dental resin-based composites. Four commercial materials (Filtek Z350 XT, Opallis, Amelogen Plus, and IPS Empress Direct) and 14 non-Vita shades were tested. Disc-shaped specimens for each resin-based composite-shade combination (n=6) were evaluated at T₀ (baseline), T₁ (after 30 days of storage in water), and T₂ (after 30 days of storage each in water and a coffee solution). Color measurements were performed

according to the L'C'h' color system. Translucency Parameter (TP) and CIEDE2000 color difference (ΔE_{00}) were calculated. Data were statistically analyzed at $\alpha = 0.05$. Baseline TP values varied from 43 ± 1 to 55 ± 1 . Changes in TP at T₁ varied from -3.0% (Opallis T-Neutral) to 4.2% (Amelogen Plus Trans Orange), with no major differences from T₀. At T₂, most resin-based composites showed significantly increased opacity, with changes varying between -15.0% (Empress Direct Trans 20) and -2.7% (Z350 XT Blue). However, the TP values were ≥ 40 throughout the study. Storage in water caused negligible color differences, with ΔE_{00} values at T₁ $\leq 0.9 \pm 0.6$. At T₂, all materials tested showed significant color difference, and $\Delta E_{00} \geq 3.2 \pm 0.2$. The orange shades from Opallis and Amelogen Plus showed lower color variation than did the other shades. The most significant optical changes upon storage were detected in the hue and particularly the chroma color coordinate. In conclusion, the high-translucent resin-based composites showed large variability in the stability of their optical properties among the tested brands and different shades of the same material. Regardless of the storage condition, the tested resin-based composites retained their high-translucency character over time.

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INTRODUCTION

The color stability of dental esthetic restoratives is of the utmost importance for their long-term perfor-

mance. Resin-based composites are known for not having the same color and optical stability as dental ceramics during clinical service.^{1,2} This has been explained by the intrinsic characteristics of dental polymers, which are more hydrophilic because of the presence of organic components that include ester linkages. After the restoration is complete, the humidity of the oral environment causes water from the saliva to penetrate and soak the restorative. With time, the water may impose hygroscopic and hydrolytic effects to different extents in the resin-based composites depending on their formulation, including their organic matrix components, inorganic filler loading, and particle size.³⁻⁶

It has been previously reported⁷ that intrinsic discoloration of dental resin-based composites may occur as a result of the oxidation of unreacted monomers and polymerization promoters that are entrapped in the polymer network, which modifies the optical properties over time. Therefore, consumption of the polymerization promoters is crucial, although a previous study⁸ found no correlation between the amount of remaining C=C and the color instability in resin-based composites. Water diffusion inward and outward may be accompanied by ions and small pigments, which can deposit on the polymer network and cause extrinsic staining. In addition, the interface between the filler particles and polymer matrix is susceptible to hydrolysis, which can contribute to optical instability through modification of the light-scattering characteristics of the surface particles.⁹ The dietary and oral hygiene habits of the patient significantly affect the presence of pigments in the mouth, the mechanical and chemical degradation of the restorative,¹⁰ and the overall color stability of the resin-based composites.

As a result of increasing esthetic demand and technical improvements in the fabrication of resin-based composite restorations in anterior teeth, high-translucency composite shades are becoming increasingly popular. Translucency refers to the passage of light through a material with associated light dispersion and is different from transparency. High-translucent resin-based composites are usually not available in conventional Vita shades. These resin-based composites are used to mimic the natural translucency and optical effects of dental tissues in the fabrication of anterior opalescent walls and lingual tooth faces. They can also be used in between layers of more chromatic resin-based composite shades to yield optical effects such as amber or blue hues in transmitted light. However, little is known about the optical properties of these materi-

als. A previous study³ reported that bisphenol A diglycidyl ether dimethacrylate (Bis-GMA)-based composites are more translucent than urethane dimethacrylate (UDMA)- and triethylene glycol dimethacrylate (TEGDMA)-based materials. Another report¹¹ observed that bleached resin-based composite shades became slightly more opaque after ageing. However, the literature lacks studies on the stability of the optical properties of high-translucency resin-based composites. For instance, it is unknown whether they retain their translucent character upon ageing. A previous study¹² showed that the less chromatic resin-based composite shades tend to have lower color stability than do the more chromatic shades. It is possible that the highly translucent character may compromise the optical stability, since pigments and oxidized unreacted species may become more apparent in the bulk of the restoration.

The aim of this study was to investigate the stability of the optical properties of high-translucent shades of dental resin-based composites. Four commercial materials and 14 high-translucent non-Vita shades were evaluated. The research hypotheses were the following: 1) ageing negatively affects the color stability; and 2) the resin-based composites would retain their highly translucent character over time.

METHODS AND MATERIALS

Study Design and Materials Tested

This *in vitro* study evaluated the influence of storage conditions (30 days in water or 30 days each in water and a coffee solution) on the optical properties of four commercially available high-translucency resin-based composites. In total, 14 groups were tested, as multiple high-translucent shades were available for each resin-based composite. Table 1 lists the composition and characteristics of all the materials tested. The sample size was calculated based on the translucency data reported in a previous study,¹³ considering a comparative design of four independent groups, an average minimum detectable difference in the means of 1.05, an expected standard deviation in the residuals of 0.4, a power of test of 0.8, and $\alpha = 0.05$. An extra specimen was added to the calculation, bringing the final sample size to six per group. The color measurements for each specimen were carried out 24 hours after photoactivation (baseline, control for each group) and repeated after the two storage periods. The response variables were color difference (ΔE_{00}), calculated by the CIEDE2000 method,^{11,12} and translucency parameter (*TP*).¹⁴

Table 1: Composition of the Commercially Available Resin-based Composites ^a					
	Shade	Resin Phase	Inorganic Phase, wt% (vol%)	Particle Size Range, μm (Mean)	Photoinitiator System
Filtek Z350 XT (3M ESPE, St Paul, MN, USA)	Blue Clear Gray Amber	UDMA, Bis-EMA, Bis-GMA, PEGDMA, TEGDMA	72.5 (55.6)	0.6-1.4 (n.a.)	CQ/amine
Amelogen Plus (Ultradent, South Jordan, UT, USA)	Trans Gray Trans White Trans Orange	Bis-GMA, TEGDMA	76 (61)	n.a. (0.7)	CQ/amine
Opallis (FGM, Joinville, SC, Brazil)	T-Blue T-Neutral T-Yellow T-Orange	Bis-GMA, Bis-EMA, TEGDMA, UDMA	78.5-79.8 (57-58)	0.04-2.0 (0.5)	CQ/amine
IPS Empress Direct (Ivoclar Vivadent, Schaan, Liechtenstein)	Trans 20 Trans Opal Trans 30	UDMA, TCDDMA, Bis-GMA	49-57 (34-36)	0.04-3 (0.55)	CQ/amine + Lucirin TPO
Abbreviations: Bis-EMA, bisphenol A ethoxylated dimethacrylate; Bis-GMA, bisphenol A glycidyl dimethacrylate; PEGDMA, polyethylene glycol dimethacrylate; TCDDMA, tricyclodecane dimethanol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate. ^a Information contained in material safety data sheets. n.a. indicates that information is not available.					

Preparation of Specimens

Six disc-shaped specimens were prepared for each resin-based composite using a cylindrical metal mold (6-mm inner diameter, 1.5-mm thickness). After the resin-based composite was inserted into the mold, the top surface was covered with a Mylar strip and flattened by pressing down with a glass slab using light pressure. The specimens were photoactivated according to the recommendations of the manufacturers from the top surface using a large spectrum LED curing unit (Bluephase G2; Ivoclar Vivadent, Schaan, Liechtenstein) operated in the high mode with 1200 mW/cm² irradiance, which was monitored throughout the study. All of the specimens were polished on both surfaces using a mechanical polisher (Buehler, Lake Bluff, IL, USA) with 2000- and 3000-grit silica carbide abrasive papers under continuous water cooling.¹⁵ The thickness of each specimen was measured with a digital caliper with 0.01-μm resolution (Digimatic Caliper; Mitutoyo, Tokyo, Japan).

Optical Data

Color measurements of the specimens were performed according to the *L'C'h'* color system, in the SCI mode,¹⁶ over a zero-calibrating box using a spectrophotometer equipped with an integrating sphere and diffuse illumination from xenon lamps (SP60; X-rite, Grand Rapids, MI, USA). The measuring aperture was 6 mm in diameter. The illumination and viewing configurations complied with the CIE 10° observer geometry and D65 illuminant.¹⁷ The analyses were performed placing the specimens against

three different backgrounds: gray (*L**=52.2, *a**=0.4, *b**=−4.6), white (*L**=89.6, *a**=0, *b**=1.3), and black (*L**=14.6, *a**=0.9, *b**=−3.2). The color measurements were performed in triplicate at the center of the top surface of each specimen and the average was used. A water-based glycerin gel was interposed between the specimen and backgrounds to maintain the optical continuity between the translucent material and the opaque backings. The *L**, *a**, and *b** values were automatically provided by the spectrophotometer. In the *L*a*b** color system, *L** represents the lightness, where 100 is white and 0 is black, and *a** and *b** represent the red-green and yellow-blue chromaticity coordinates, respectively. In the *L'C'h'* color system, *L'* represents the lightness, *C'* the chroma, and *h'* the hue. They were calculated using the following formula¹⁸:

$$L' = L^*, C' = \sqrt{a^{*2} + b^{*2}}, \text{ and } h' = \tan^{-1}(b'/a')$$

Considering

$$a' = (1 + G)a^*, b' = b^*, \text{ and } G = \left(1 - \sqrt{\frac{C^*}{C^{*7} + 25^7}}\right)$$

Color Stability

The color measurements for all materials were performed in three different periods. The baseline values (*T*₀ = control) were taken 24 hours after the polymerization of the specimens, which were dry-stored in the dark. The specimens were then immersed in distilled water at 37°C for 30 days and

the color measurements were repeated (T_1). The final measurements were performed after the immersion of the specimens in a coffee solution at 37°C for 30 days (T_2). The coffee solution was prepared according to the method described in a previous report.¹⁹ The immersion medium was not renewed during storage. Color stability was estimated by the CIEDE2000 color difference (ΔE_{00}), using the color coordinates measured over the gray background, calculated according to the following formula^{17,20}:

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L} \right)^2 + \left(\frac{\Delta C'}{K_C S_C} \right)^2 + \left(\frac{\Delta H'}{K_H S_H} \right)^2 + R_T \left(\frac{\Delta C'}{K_C S_C} \right) \left(\frac{\Delta H'}{K_H S_H} \right) \right]^{\frac{1}{2}}$$

by considering

$$\Delta L' = L'_1 - L'_2, \Delta C' = C'_1 - C'_2,$$

$$\Delta H' = \sqrt{C'_1 C'_2 \sin\left(\frac{\Delta h'}{2}\right)}, \text{ and } \Delta h' = h'_1 - h'_2$$

where $\Delta L'$, $\Delta C'$, and $\Delta H'$ are the mathematical differences in lightness, chroma, and hue, respectively, between the two measurement periods (ie, water storage vs baseline/ T_1 - T_0 ; and water + coffee storage vs baseline/ T_2 - T_0). The rotation term R_T is a function that accounts for the interaction between chroma and hue differences in the blue region, improving the color difference equation performance. For dental color space, the R_T value is close to zero.²¹ $K_L S_L$, $K_C S_C$, and $K_H S_H$ are empirical terms used for weighting the metric differences to the CIEDE2000 differences for each coordinate. Parametric factors were set to $K_L = 2$, $K_H = 1$, and $K_C = 1$ according to the better performance of the ΔE_{00} equation.^{22,23} During ΔE_{00} calculation, the discontinuities due to mean hue computation and hue difference were taken into account, as pointed out previously.²⁰

Translucency

Translucency of each specimen was calculated using the TP formula, thus¹⁴:

$$TP = [(L^*_w - L^*_b)^2 + (a^*_w - a^*_b)^2 + (b^*_w - b^*_b)^2]^{1/2}$$

where w refers to the CIELAB values for each specimen measured over the white background and b over the black background. The TP was calculated for each storage period.

Statistical Analysis

The analyses were conducted using Sigma Plot v13.0 software (Systat Software, San Jose, CA, USA). Data for individual color coordinates L' , C' , and h' , TP , and ΔE_{00} were analyzed using two-way repeated-measures analysis of variance (shade vs ageing condition), with one-factor repetition. Comparisons between the different resin-based composites were not performed. All pairwise multiple comparison procedures were performed using the Student-Newman-Keuls post hoc method. The analyses were performed at $\alpha = 0.05$.

RESULTS

Translucency

Results for TP are shown in Table 2. For all resin-based composites tested, the factors of shade ($p < 0.01$) and ageing condition ($p < 0.001$) were significant, whereas the interaction between the two factors was not significant ($p \geq 0.08$), except for Amelogen Plus ($p = 0.031$). Changes in TP after 30 days of water storage varied from -3.0% (Opallis T-Neutral) to 4.2% (Amelogen Plus Trans Orange). In general, the resin-based composites showed no significant differences in translucency between T_0 and T_1 , except for Amelogen Plus Trans Orange, which became significantly more translucent. After storage in coffee, changes in TP were always negative (increased opacity), varying between -15.0% (Empress Direct Trans 20) and -2.7% (Z350 XT Blue). A significant decrease in TP was observed at T_2 for all resin-based composites except the shades Blue and Amber (Z350 XT) and Trans Gray (Amelogen Plus). The material with the least stable TP upon storage was Amelogen Plus Trans Orange, which showed significant differences in TP in all storage periods. When the different shades were compared within each resin-based composite, the TP was significantly lower for the Blue shade from Z350 XT, the T-Neutral shade from Opallis, and the Trans 20 shade from Empress Direct. The resin-based composite Amelogen Plus showed negligible differences in TP among the different shades.

Color Stability

Results for ΔE_{00} are shown in Table 3. For all resin-based composites except Z350 XT, the factors shade ($p \leq 0.014$) and ageing condition ($p < 0.001$) were significant, as was the interaction between the two factors ($p < 0.001$). For Z350 XT, the ageing condition was the only significant factor ($p < 0.001$). After storage in water (T_1), all resin-based composites

Table 2: Translucency Parameter (TP) Means $\pm$ Standard Deviations and Percentage Alteration in TP (Δ TP) Upon Ageing ($n=6$)^a

Shade		Ageing Condition			Δ TP	
		T ₀	T ₁	T ₂	T ₁ vs T ₀ , %	T ₂ vs T ₀ , %
Filtek Z350 XT (3M ESPE)	Blue	47.4 $\pm$ 0.8 A,b	48.0 $\pm$ 0.9 A,b	46.1 $\pm$ 1.3 A,b	1.3	-2.7
	Clear	52.6 $\pm$ 0.5 A,a	54.4 $\pm$ 2.2 A,a	49.8 $\pm$ 1.9 B,a	3.4	-5.3
	Gray	51.6 $\pm$ 1.1 A,a	52.0 $\pm$ 1.3 A,a	49.4 $\pm$ 1.8 B,a	0.8	-4.3
	Amber	52.5 $\pm$ 0.7 A,a	52.6 $\pm$ 2.0 A,a	48.7 $\pm$ 2.0 A,a	0.2	-7.2
Opallis (FGM)	T-Blue	50.0 $\pm$ 0.5 A,b	49.7 $\pm$ 1.0 A,b	47.7 $\pm$ 2.1 B,b	-0.6	-4.6
	T-Neutral	46.5 $\pm$ 3.2 A,c	45.1 $\pm$ 0.9 A,d	41.3 $\pm$ 1.2 B,c	-3.0	-11.2
	T-Yellow	54.6 $\pm$ 1.1 A,a	54.4 $\pm$ 0.8 A,a	50.4 $\pm$ 0.9 B,a	-0.4	-7.7
	T-Orange	47.5 $\pm$ 1.9 A,c	47.4 $\pm$ 0.5 A,c	43.3 $\pm$ 2.1 B,c	-0.2	-8.8
Amelogen Plus (Ultradent)	Trans Gray	44.8 $\pm$ 1.4 A,a	44.7 $\pm$ 1.4 A,b	41.9 $\pm$ 1.5 A,a	-0.2	-6.5
	Trans White	46.1 $\pm$ 1.5 A,a	46.9 $\pm$ 1.2 A,a	43.4 $\pm$ 1.5 B,a	1.7	-5.9
	Trans Orange	44.9 $\pm$ 1.1 B,a	46.8 $\pm$ 1.1 A,a	39.9 $\pm$ 1.3 C,a	4.2	-11.1
IPS Empress Direct (Ivoclar Vivadent)	Trans 20	43.4 $\pm$ 1.0 A,c	45.2 $\pm$ 3.0 A,b	36.9 $\pm$ 1.3 B,b	4.1	-15.0
	Trans Opal	49.9 $\pm$ 1.2 A,b	50.3 $\pm$ 1.0 A,a	44.4 $\pm$ 0.9 B,a	0.8	-11.0
	Trans 30	52.7 $\pm$ 1.6 A,a	51.2 $\pm$ 1.3 A,a	45.6 $\pm$ 2.4 B,a	-2.8	-13.5

^a T₀, baseline; T₁, 30 days of water storage; T₂, 30 days of water storage + 30 days of coffee storage. For each resin-based composite, distinct capital letters in the same line indicate significant differences between the ageing conditions, and distinct lowercase letters in the same column indicate significant differences between the shades ($p < 0.05$). Comparisons between the different resin-based composites were not performed.

showed negligible color differences, with ΔE_{00} values below 1.25, which has been reported²¹ to correspond to 50% of visual perceptibility under clinical conditions and is herein used as a reference for the visually detectable color changes. After storage in coffee solution (T₂), all materials tested showed significant color difference compared with water storage. There were significant differences between

the different shades for some resin-based composites, but all ΔE_{00} values were above 1.25. The orange shades from both Opallis and Amelogen Plus showed lower color variation than did the other shades.

L', C', and h' Color Coordinates

Results for lightness (L'), chroma (C'), and hue (h') are presented in Figure 1. The L' coordinate was not

Table 3: CIEDE2000 Color Difference (ΔE_{00}) Means $\pm$ Standard Deviations ($n=6$)^a

Shade		Ageing Condition	
		T ₁ vs T ₀	T ₂ vs T ₀
Filtek Z350 XT (3M ESPE)	Blue	0.6 $\pm$ 0.3 B,a	4.0 $\pm$ 0.2 A,b
	Clear	0.6 $\pm$ 0.1 B,a	4.9 $\pm$ 0.6 A,a
	Gray	0.5 $\pm$ 0.1 B,a	4.4 $\pm$ 0.4 A,ab
	Amber	0.5 $\pm$ 0.2 B,a	4.3 $\pm$ 0.3 A,ab
Opallis (FGM)	T-Blue	0.7 $\pm$ 0.2 B,b	4.8 $\pm$ 0.7 A,a
	T-Neutral	0.6 $\pm$ 0.2 B,b	5.5 $\pm$ 0.7 A,a
	T-Yellow	0.9 $\pm$ 0.2 B,a	5.0 $\pm$ 0.5 A,a
	T-Orange	0.8 $\pm$ 0.2 B,ab	3.5 $\pm$ 0.6 A,b
Amelogen Plus (Ultradent)	Trans Gray	0.6 $\pm$ 0.2 B,a	5.6 $\pm$ 0.6 A,a
	Trans White	0.7 $\pm$ 0.4 B,a	5.6 $\pm$ 0.4 A,a
	Trans Orange	0.9 $\pm$ 0.6 B,a	3.2 $\pm$ 0.2 A,b
IPS Empress Direct (Ivoclar Vivadent)	Trans 20	0.5 $\pm$ 0.3 B,a	8.8 $\pm$ 0.3 A,b
	Trans Opal	0.5 $\pm$ 0.2 B,a	10.0 $\pm$ 0.6 A,a
	Trans 30	0.5 $\pm$ 0.2 B,a	8.6 $\pm$ 0.6 A,c

^a T₀, baseline; T₁, 30 days of water storage; T₂, 30 days of water storage + 30 days of coffee storage. For each material, distinct capital letters in the same line indicate significant difference between the ageing conditions, and distinct lowercase letters in the same column indicate significant difference between the shades ($p < 0.05$). Comparisons between the different resin-based composites were not performed.

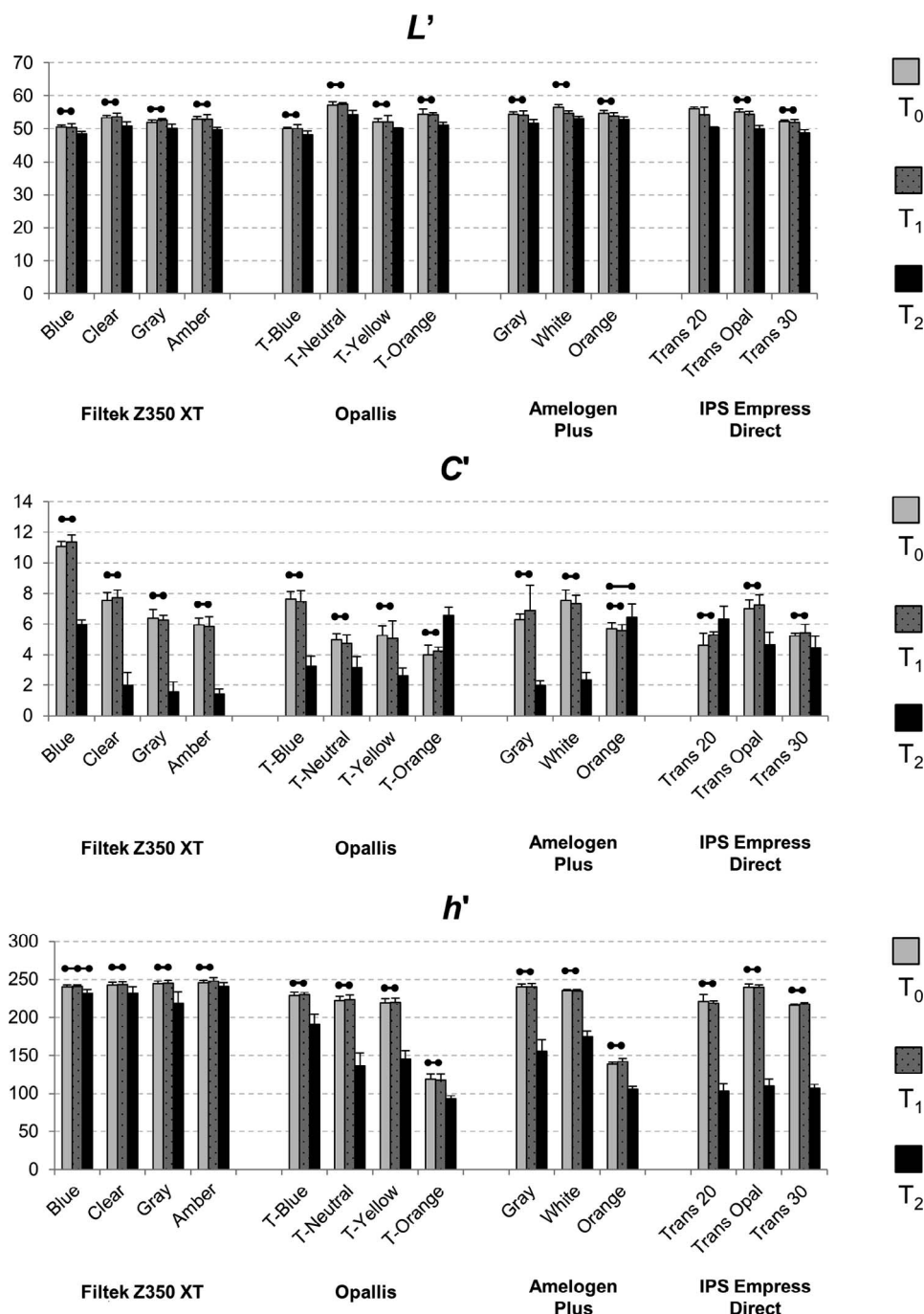


Figure 1. Results for lightness (L'), chroma (C'), and hue (h') of all resin-based composites and shades tested. The bars indicate means $\pm$ standard deviations ($n=6$). The connected dots above bars for each shade indicate no significant differences between the ageing periods: T_0 , baseline; T_1 , 30 days of water storage; T_2 , 30 days of water storage + 30 days of coffee storage.

affected by water storage for most resin-based composites and shades tested, except Trans 20 (Empress Direct), which had slightly but significantly lower L' at T_1 . The lightness was significantly lower for all materials after storage in coffee (T_2). Significant changes upon storage were detected in the C' coordinate, particularly at T_2 . A significant reduction in C' was observed for all shades from Z350 XT and for most shades from Opallis and

Amelogen Plus, except the orange shades, which showed significantly higher C' after storage in coffee. For Empress Direct, the changes in C' at T_2 tended to be less pronounced compared with the changes associated with the other resin-based composites. For the h' coordinate, the changes upon storage were negligible for Z350 XT, whereas the other resin-based composites showed significant reductions in h' at T_2 .

DISCUSSION

This study is the first to investigate the color stability of high-translucency resin-based composite shades. To the best of our knowledge, no previous reports on the optical properties of high-translucent resin-based composites can be found in the literature. The baseline average *TP* values varied from 43 to 55. These values are two to seven times higher than those reported in the literature for conventional, chromatic resin-based composites, which have *TP* values ranging between 8 and 21 for specimens with 1.0 to 1.5 mm in thickness.^{10,16,18} This suggests that the materials tested here have high translucency and are suitable for use in anterior restorations when the clinician desires optical effects.

The resin-based composites tested herein are commercially available in shades that are not compatible with the conventional Vita shade guide, and the same manufacturer usually offers different shades. In this study, differences in translucency were observed between resin-based composite brands and within shades of the same resin-based composite. These findings can be explained by the presence of pigments and other components added to tune the optical effects of these resin-based composites, which are available in orange, yellow, blue, and other shades. The translucency of dental resin-based composites is determined by macroscopic phenomena such as monomer characteristics, particle composition, and filler content, as well as by relatively minor pigment additions and potentially by all other chemical components.^{13,14} Different materials and shades are likely to exhibit minor changes in composition to achieve the optical effects intended by the manufacturer. These minor changes may lead to significant changes in translucency. However, even after storage in coffee solution for 30 days, yielding significant changes in the individual color coordinates and overall stability, the restoratives retained their highly translucent character. Thus, the tested hypotheses could not be rejected.

The effects of water storage on color stability are likely to be related to the leaching or oxidation of the unreacted polymerization promoters and monomers, since longer ageing times are needed to observe polymer degradation effects.²² In general, water ageing caused negligible changes in *TP*, lightness, chroma, and hue for most resin-based composites, but there were few exceptions. Resin-based composites from different manufacturers vary in the concentration and structure of their photoinitiators and reducing amines. These differences are also present between different non-Vita shades from the

same resin-based composite brand. Variations in the formulation of the resin phase and polymer swelling characteristics may have implications for color stability during water storage. The refractive index of the polymer is different from that of water, altering the light transmission after water accesses the polymer structure and leading to changes in color perception.²³ From a clinical standpoint, these results suggest that the actual optical characteristics of a resin-based composite restoration can only be determined after a certain amount of intraoral ageing. In the ageing process, the restorative is soaked by water and unreacted polymerization promoters or monomers dissolve or are removed.

The color and translucency alterations caused by ageing in coffee were more pronounced than those effects caused by water. The effects of coffee are related to the adsorption pigments and their deposition in the polymer structure. Changes at T_2 are a summation of both water and coffee storage, since the specimens were exposed to the coffee solution after ageing in water for 30 days. Storage in coffee caused a slight decrease in L' and an overall reduction in *TP*. It has been reported²⁴ that the translucency of resin-based composites is mainly influenced by changes in lightness. Because it is a dark solution, coffee possesses a large amount of pigments that may deposit into the bulk of the restorative, absorb more light, and increase light diffusion, resulting in increased opacity.²⁵ However, the resin-based composites were still able to retain their high-translucency character over time. The most appreciable changes from coffee exposure were related to hue and particularly chroma. A previous study¹² reported that exposure to coffee may cause dental resin-based composites to redden. However, the discoloration by coffee is often attributed to its yellow pigments.²⁶ The polarity compatibility between the polymer and coffee colorants likely facilitates their incorporation into the restorative. These findings are in agreement with those of previous studies^{24,27} that compared the behavior of resin-based composites after immersion in different beverages and observed that beverages with yellowish pigments were more likely to cause significant color alterations.

The various resin-based composites and shades tested here have differences in inorganic filler loadings. With higher filler content, less resin phase is present, and it would be expected that the resin-based composites with lower particle contents exhibit higher optical instability. The resin-based composite Empress Direct presented lower translu-

gency stability than did the other restoratives and the highest ΔE_{00} values upon storage. Empress Direct has the lowest filler content among all restoratives tested. In addition, it presents a different photoinitiator system. This higher optical instability could be a result of increased water sorption, leaching and oxidation of unreacted species, and the higher absorption of pigments.⁵ A recent study investigating the degradation of the optical and surface properties of resin-based composites indicated that other factors may also influence their ageing resistance, such as the surface area of the fillers and the quantity and type of the coupling agent used to bond the particles with the resin phase.⁶ As mentioned previously, the color and translucency stability of the resin-based composite are likely affected by all the components of the material, including the organic matrix formulation, type and quantity of coupling agent, as well as the type and size of the reinforcing inorganic particles.^{8,26} Polymeric characteristics may also interfere with the performance of resin-based composites upon ageing, with the degree of C=C conversion and crosslink density of the network having the most significant influences. Finally, the orange shades from two resin-based composites tested herein showed lower color difference than the other shades and an increase in chroma upon storage. This finding could be explained by the similar color between the coffee pigments and the orange shades, reducing the overall staining effects.

In the oral environment, dental resin-based composite restorations are subjected to many chemical and physical challenges that together may significantly influence the stability of their optical properties during clinical service. It has been reported that the surface free energy of resin-based composites is significantly increased by water storage and that saliva coating may influence the physicochemical surface characteristics of resin-based composites, including their surface charge and hydrophobicity.¹³ Therefore, surface characteristics may have significant implications in terms of the retention of pigments and their subsequent adsorption by the polymer. The translucency is mainly influenced by the scattering of light at multiple scattering centers within the material, such as the opacifiers or pigments,²⁸ and may change as a function of the wavelength of the incident light.¹⁴ In the case of high-translucent resin-based composites, the present study indicates that they were able to maintain their high-translucency character upon ageing, but large variations in the optical properties and stability

were observed among the different materials. A systematic review highlighted that even different types of finishing and polishing systems may affect the maintenance of surface smoothness and gloss of the chromatic resin-based composites over time.²⁹ These findings suggest that close follow up of the patients who received resin-based composite restorations would be beneficial for the study of the stability of the optical properties of the treatments.

CONCLUSIONS

Within the scope of this *in vitro* study, the following conclusions can be drawn:

- High-translucent resin-based composites showed large variability in the stability of their optical properties among the resin-based composite brands and different shades of the same material;
- Ageing in water had minor effects on color and translucency stability, whereas ageing in coffee had a more significant impact on the optical properties;
- The chroma and hue color coordinates experienced greater effects than did lightness on color alteration; and
- Regardless of the storage condition, the tested resin-based composites retained their high-translucency characteristics over time.

Acknowledgement

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Bond Strength Stability of Self-adhesive Resin Cement to Etched Vitrified Yttria-stabilized Tetragonal Zirconia Polycrystal Ceramic After Thermomechanical Cycling

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Clinical Relevance

Hydrofluoric acid etching of yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) ceramic surfaces vitrified with low-fusing glaze withstood thermomechanical cycling. This surface treatment is suitable for improving the bonding stability of resin cements to Y-TZP ceramics.

SUMMARY

The purpose of this study was to evaluate the influence of thermomechanical cycling on the bond strength stability of self-adhesive resin

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cement to vitrified yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) ceramic. Y-TZP ceramic blocks were divided into four groups according to the surface treatments: AS, as sintered; SB, sandblasted with 50- μ m Al_2O_3 particles; LG, vitrification with a low-fusing glaze and etching with 10% hydrofluoric acid; and HC, vitrification with a ceramic liner and etching with 10% hydrofluoric acid. Lithium disilicate ceramic blocks etched with 10% hydrofluoric acid for 20 seconds (EM) were

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also tested. Blocks of resin composite were cemented on the ceramic block surfaces using a self-adhesive resin cement. Half of the blocks from each group were cut into beams and submitted to microtensile bond strength (μ TBS) testing after immersion in water at 37°C for 24 hours and the other half after mechanical (1,200,000 cycles, 98 N, 2.5 Hz) and thermal (10,000 cycles, 5-55°C, 30-second dwell time) cycling (TMC). Data were analyzed using one-way analysis of variance, Tukey's honest significant difference post hoc test, and Student *t*-test ($\alpha=0.05$). After 24 hours, EM and LG presented similar and the highest μ TBS, whereas AS showed the lowest μ TBS ($p<0.05$). After TMC, EM presented the highest μ TBS, followed by LG ($p<0.05$). Only EM and LG maintained the stability of μ TBS after TMC ($p>0.05$). The bonding to Y-TZP ceramic vitrified with a low-fusing glaze withstood thermo-mechanical cycling.

INTRODUCTION

Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) ceramics were introduced to replace metallic frameworks in full crowns, implant abutments, and dental bridges in anterior and posterior areas for esthetic oral rehabilitation.¹ In addition to its esthetic appearance, the principal advantage of Y-TZP ceramic is a stress-induced toughening mechanism that is started by the stresses generated at the ceramic surface. As a consequence, a tetragonal to monoclinic phase transformation occurs, along with a volume increase (3%-5%) at the crack origins, thereby developing internal stresses that counterattack crack propagation inside the ceramic bulk and increase the mechanical strength of the ceramic.² Irrespective of this welcome mechanism, the absence of a vitreous phase, which makes Y-TZP ceramics unsusceptible to hydrofluoric acid etching, still represents a point of weakness for the clinical performance of this class of restorative biomaterial.³⁻⁶

In an attempt to overcome this limitation, different strategies have been proposed to improve the interaction of resin cements to Y-TZP ceramic surfaces, such as treatment with phosphate adhesive monomers,^{7,8} sandblasting with Al_2O_3 and Si-modified Al_2O_3 particles,⁹⁻¹¹ treatment with selective primers,^{12,13} Si nanocoating,^{14,15} and the application of nonthermal plasmas.¹⁶⁻¹⁸ Although these protocols can increase the immediate bond strength of resin cements to Y-TZP ceramics, a drop in bond

strength can occur when submitted to long-term water immersion and thermal or mechanical cycling.^{4,9}

Because of a possible chemical interaction at the interfacial grain level between the phosphate groups present in methacrylate phosphoric acid esters and the hydroxyl groups of the passive ZrO_2 coating on the Y-TZP ceramic, self-adhesive resin cements have been advocated for their better adherence to this type of ceramic.⁸ Unfortunately, scientific evidence suggests that this mechanism can also suffer degradation when submitted to aging methods,^{9,19} which are clinical matters of concern.

A new approach for optimizing the bonding of resin cements to Y-TZP ceramics has recently presented successful results, even after aging in water and thermocycling. This protocol involves the modification of the ceramic surface with low-fusing glasses or ceramic liners, which is a vitrification process itself. This mechanism creates a silica-rich layer on the Y-TZP ceramic surface that is etchable by hydrofluoric acid, thereby favoring micromechanical interlocking with the resin cement and a chemical interaction with the silane-coupling agent as well.²⁰⁻²⁶

Although these former studies have presented interesting results, it is noteworthy that none of them had submitted the bond interfaces to mechanical loading. From a clinical point of view, the mouth is a dynamic system in which Y-TZP ceramic restorations are subject to changes in temperature and masticatory loading.²⁷ Thus, it seems of some relevance to investigate the effect of thermomechanical cycling on the bond strength of resin cements to a Y-TZP ceramic surface previously submitted to vitrification. Therefore, the purpose of the current study was to evaluate the influence of thermomechanical cycling on the bond strength of self-adhesive resin cement to Y-TZP ceramic submitted to surface vitrification. The tested hypothesis was that thermomechanical cycling would not affect the bond strength of self-adhesive resin cement to vitrified Y-TZP ceramic.

METHODS AND MATERIALS

Microtensile Bond Strength Evaluation

The experimental setup of ceramic-resin composite beam preparation and microtensile bond strength (μ TBS) testing is illustrated in Figure 1. Forty presintered blocks of a Y-TZP ceramic (Lava Frame, 3M ESPE, Seefeld, Germany) and 10 blocks of a lithium disilicate glass ceramic (IPS e.MAX CAD,

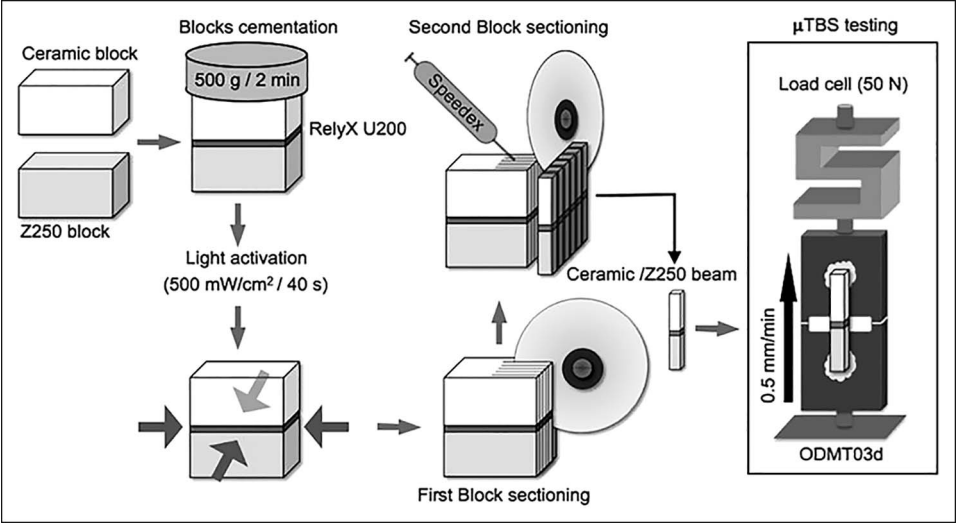


Figure 1. Experimental setup of ceramic-resin composite beam preparation and microtensile bond strength testing (μ TBS).

Ivoclar Vivadent AG, Schaan, Liechtenstein) were used in the present study. After sectioning with a diamond disk, at 800 rpm, in a precision sectioning cutter (Isomet 1000 precision saw, Buehler, Lake Bluff, IL, USA), the blocks were sintered and crystallized using the electrical induction furnaces recommended by the manufacturers: Lava Furnace 200 (3M ESPE, St Paul, MN, USA) and Programat P310 (Ivoclar Vivadent AG). After these processes, the final dimensions of all ceramic blocks were 10.0 mm $\times$ 10.0 mm $\times$ 7 mm. After polishing with 600-grit SiC paper, the blocks were ultrasonically cleaned for five minutes in distilled water and divided into five groups (n=10) according to surface treatment (Table 1).

Fifty blocks of a resin composite (Filtek Z250, 3M ESPE) were built up using a plastic mold with the same dimensions as the ceramic blocks (10.0 $\times$ 10.0 $\times$

7.0 mm). The mold was filled with 2.0-mm-thick increments of Z250, which were individually light polymerized for 30 seconds each with an irradiance of 500 mW/cm² (Radii call, SDI, Victoria, Australia). Afterward, the blocks were polished with 600-grit SiC paper to standardize the roughness and were then ultrasonically cleaned for five minutes in distilled water.²⁸

A self-adhesive resin cement (RelyX U200, 3M ESPE) was used to bond the resin composite blocks to the ceramic blocks. Equals amounts of base and catalyst pastes of RelyX U200 were mixed with a Teflon spatula on a mixing pad for 10 s. After the resin cement had been applied on the ceramic surface, the resin composite block was placed on the cement and held under a mass of 500 g for two minutes to standardize the resin cement pellicle thickness for all specimens. Afterward, the resin

Table 1: Ceramic Surface Treatments	
Group	Treatment
EM	IPS e.max CAD ceramic etched with 10% hydrofluoric acid for 20 s, rinsed with air-water spray, dried, and ultrasonically cleaned in distilled water for 5 min
AS	As-sintered Y-TZP ceramic with no treatment
SB	Y-TZP ceramic sandblasted with 50- μ m Al ₂ O ₃ particles for 20 s at a perpendicular distance of 10 mm at 2.8 bar pressure and ultrasonically cleaned in distilled water for 5 min
LG	Y-TZP ceramic vitrified with a low-fusing ceramic glaze (*VITA Akzent Plus spray, VITA Zahnfabrik, Bad Sackingen, Germany; applied twice) sintered following the manufacturer's guideline, etched with 10% hydrofluoric acid for 1 min, rinsed with air-water spray, dried, and ultrasonically cleaned in distilled water for 5 min *(SiO ₂ , K ₂ O, Na ₂ O, Al ₂ O ₃ , isobutane, ethanol)
HC	Y-TZP ceramic vitrified with a ceramic liner (**HeraCeram Zirkonia Liner, Heraeus Kulzer, Hanaus, DE; one-layer brushing), sintered for 10 min at 1050°C, etched with 10% hydrofluoric acid for 1 min, rinsed with air-water spray, dried, and ultrasonically cleaned in distilled water for 5 min **(SiO ₂ , Al ₂ O ₃ , K ₂ O, CeO ₂)
Abbreviations: AS, as sintered; EM, etched with 10% hydrofluoric acid for 20 seconds; HC, vitrification with a ceramic liner and etching with 10% hydrofluoric acid; LG, vitrification with a low-fusing glaze and etching with 10% hydrofluoric acid; SB, sandblasted with 50- μ m Al ₂ O ₃ particles; Y-TZP, yttria-stabilized tetragonal zirconia polycrystal.	

cement was light activated from the four sides of the Y-TZP ceramic-resin composite bonded interface for 40 seconds each with an irradiance of 500 mW/cm² (Radii call, SDI).

After storage in distilled water at 37°C for 24 hours, half of the Y-TZP ceramic-resin composite blocks were attached to a cutting machine (IsoMet 1000, Buehler) and longitudinally sectioned, across the bonded interfaces, in both *x*- and *y*-axes, using a water-cooled diamond disc (Isomet Wafering Blade 15LC #114254, 0.3-mm thickness, Buehler) at 800 rpm, thus producing beams with a cross-sectional area of approximately 1.0 mm² and 10.0 mm in length. To avoid premature loss of beams during the ceramic-resin composite block cutting, wash silicone impression material (Speedex, Vigodent-COLTENE, RJ, Brazil) was injected between the slices produced during the first cutting to absorb the vibration generated during the second cutting. Afterward, each beam was attached to a μ TBS device (ODMT03d, Odeme Dental Research, Luzerna, SC, Brasil) using cyanoacrylate adhesive (SuperBonder, lot No. 15900545, Henkel Ltda, Itapevi, SP, Brazil) and loaded in tension using a universal testing machine (Emic DL 2000, São José dos Pinhais, SP, Brazil), and a 50-N load cell, at a crosshead speed of 0.5 mm/min, until failure occurred. The μ TBS (MPa) was obtained by dividing the load at failure (N) by the cross-sectional area of the beam (mm²).

Before the μ TBS test, the other half of the blocks were submitted to thermomechanical cycling as follows: first, the blocks were submitted to mechanical cycling in a chewing simulator (ER-37000NG, ERIOS, São Paulo, SP, Brazil), with a load of 98 N and a frequency of 2.5 Hz for 1.2×10^6 cycles. The load was applied using a stainless-steel plate adapted to the piston of the chewing simulator to transmit the mechanical stresses equally to the bond interfaces. After the blocks were sectioned into beams, they were thermocycled (5°C–55°C with a dwell time of 30 seconds and transfer time of two seconds for 10×10^3 cycles). With the exception of the as-sintered (AS) group, in which all blocks spontaneously debonded during mechanical cycling, 80 beams were produced for each group, with 16 beams from the central area of each ceramic-resin composite block being used for experimentation.

Failure Mode Analysis

Each failed beam was evaluated using a stereomicroscope at 40 $\times$ magnification (SZ40, Olympus, Tokyo, Japan), and the failure mode was classified as adhesive (failures at the adhesive interfaces),

cohesive (failures occurring mainly within resin composite), or mixed (mixture of adhesive and cohesive failure within the same fractured surface). In addition, beams presenting different failure modes and with the value of μ TBS close to the mean of each group were viewed using scanning electron microscopy (SEM). The beams were mounted in a charge reduction sample holder and observed under SEM (PhenomProX, PhenomWorld, Eindhoven, the Netherlands) operating in the backscattered mode, in a low-vacuum environment. The SEM images were taken at a magnification of 270 $\times$.

Surface Topography Analysis

Two ceramic surfaces submitted to each treatment were analyzed using SEM and three-dimensional (3D) profilometry. First, the ceramic specimens were observed under SEM (Phenom ProX, Phenom World) operating in the backscattered mode under low vacuum. The images were taken with 10 kV, at magnifications of 1000 $\times$. Afterward, the topographic analysis was performed using a 3D profilometer (Form Talysurf 60i, Taylor Hobson, Leicester, UK). For each specimen, an area of 1 mm² was scanned with a 20-nm z-resolution, employing 4000 steps in the *x*-axis and a spacing of 2 μ m in the *y*-axis. The reconstruction of 3D images was made according to the parameter Sa (μ m) using the following formula:

$$Sa = \frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} |z(x_k; y_l)|,$$

where (*M*, *N*) are the number of points in (*x*, *y*), and *z* is the height of measured points at *x* and *y* coordinates.

Statistical Analysis

The obtained data were analyzed using Statgraphics Centurion XVI software (STATPOINT Technologies Inc, Warrenton, VA, USA). The sample size for the μ TBS test was calculated based on the mean and standard deviation values obtained in the pilot study and considering a statistical test power of 0.87. As all blocks in the AS group spontaneously debonded during mechanical cycling (μ TBS = 0 MPa), these data were not included in the statistical analysis.^{25,29} The prematurely failed beams³⁰ were included in the statistical analysis by assigning a value that corresponded to half of the minimum μ TBS for their experimental group.³¹ The normal distribution of errors and the homogeneity of variances were checked using Shapiro-Wilk's and Levene's test, respectively. Based on these preliminary analyses,

Table 2: Means $\pm$ Standard Deviation of μ TBS (MPa) for All Experimental Groups^a

Group	24 h	Thermomechanical Cycling
EM	37.3 $\pm$ 4.4 ^{A,a}	34.8 $\pm$ 4.7 ^{A,a}
AS	7.1 $\pm$ 2.7 ^{C,a}	0 ^{D,b}
SB	18.1 $\pm$ 2.0 ^{B,a}	12.3 $\pm$ 2.7 ^{C,b}
LG	30.6 $\pm$ 4.2 ^{A,a}	24.1 $\pm$ 3.3 ^{B,a}
HC	23.0 $\pm$ 2.6 ^{B,a}	11.6 $\pm$ 1.4 ^{C,b}

Abbreviations: AS, as sintered; EM, etched with 10% hydrofluoric acid for 20 seconds; HC, vitrification with a ceramic liner and etching with 10% hydrofluoric acid; LG, vitrification with a low-fusing glaze and etching with 10% hydrofluoric acid; SB, sandblasted with 50- μ m Al₂O₃ particles; μ TBS, microtensile bond strength.
^a Means followed by different letters (uppercase, column; lowercase, row) are statistically different (Tukey's honest significant difference, $p < 0.05$).

the μ TBS data after 24 hours and after thermomechanical cycling (TMC) were analyzed separately using one-way analysis of variance and Tukey's honest significant difference post hoc test. The effect of TMC in each group was evaluated using the Student *t*-test. The analyses were performed at a significance of $\alpha = 0.05$.

RESULTS

The means and standard deviations of μ TBS (MPa) are depicted in Table 2. After 24 hours, etching with 10% hydrofluoric acid for 20 seconds (EM; 37.3 $\pm$ 4.4) and vitrification with a low-fusing glaze and etching with 10% hydrofluoric acid (LG; 30.6 $\pm$ 4.2) presented the highest μ TBS values, with no statistical differences from each other, followed by vitrification with a ceramic liner and etching with 10% hydrofluoric acid (HC; 23.0 $\pm$ 2.6) and sandblasting with 50- μ m Al₂O₃ particles (SB; 18.1 $\pm$ 2.0; $p < 0.05$). The lowest μ TBS was presented by AS ($p < 0.05$). After TMC, EM (34.8 $\pm$ 4.7) presented the highest μ TBS ($p < 0.05$), followed by LG (24.1 $\pm$ 3.3), whereas SB (12.3 $\pm$ 2.7) and HC (11.6 $\pm$ 1.4) presented statistically similar μ TBS ($p > 0.05$). All specimens of AS spontaneously debonded during mechanical cycling. Only EM and LG maintained the stability of μ TBS after TMC.

Table 3 summarizes the number of specimens that failed prematurely and the number of specimens that were submitted to μ TBS testing. All blocks in AS-TMC spontaneously debonded during mechanical cycling. The number of prematurely failed beams in the other groups was as follows: AS-24 > SB-24 > SB-TMC > HC-TMC > HC-24 > LG-24. The groups LG-TMC, EM-24, and EM-TMC presented no prematurely failed beams.

The percentage of failure modes are described in Figure 2A. After 24 hours, EM and LG presented the

Table 3: Total (T) of Produced Beams, Number (n) and Percentage (%) of Pretesting Failures (PTF), and Total (N) Beams Submitted to μ TBS Test

Group	T	n (%) PTF	n
EM-24	80	0 (0)	80
EM-TMC	80	0 (0)	80
AS-24	80	64 (80)	16
AS-TMC	0	—	0
SB-24	80	39 (49)	41
SB-TMC	80	32 (40)	48
LG-24	80	13 (16)	67
LG-TMC	80	0 (0)	80
HC-24	80	14 (17)	66
HC-TMC	80	29 (36)	51

Abbreviations: AS, as sintered; EM, etched with 10% hydrofluoric acid for 20 seconds; HC, vitrification with a ceramic liner and etching with 10% hydrofluoric acid; LG, vitrification with a low-fusing glaze and etching with 10% hydrofluoric acid; SB, sandblasted with 50- μ m Al₂O₃ particles; TMC, thermomechanical cycling; μ TBS, microtensile bond strength.

highest percentage of cohesive failures, whereas the HC group showed equilibrium between adhesive and mixed failures. On the other hand, AS and SB presented a predominance of adhesive failures. After TMC, SB presented a predominance of adhesive failures, whereas LG, HC, and EM showed a greater percentage of mixed failures. Representative SEM images of debonded specimens are presented in Figure 2 (B, C, D, E): (B) adhesive failure of an HC specimen showing the Y-TZP ceramic surface free of composite (c) and small remnants of resin cement (rc), (C) an EM specimen showing a cohesive failure in the bulk of composite (c), (D) mixed failure in an LG specimen, and (E) mixed failure in an SB specimen showing residual resin cement (rc) and composite (c) on the Y-TZP ceramic surface.

Figure 3 shows representative SEM images of an IPS e.max CAD specimen etched with 10% hydrofluoric acid for 20 seconds (A) and Y-TZP ceramic surfaces submitted to the following treatments: (B) as sintered, (C) sandblasted with 50- μ m Al₂O₃ particles for 20 seconds, (D) vitrified with low-fusing ceramic glaze and etched with 10% hydrofluoric acid for one minute, and (E) vitrified with ceramic liner and etched with 10% hydrofluoric acid for one minute. The surfaces of the IPS e.max CAD specimen and the Y-TZP ceramic vitrified with low-fusing glaze presented a similar appearance, with disperse porous characteristics of partially dissolved glass ceramics (pointers). Typical grooves are clear in the sandblasted Y-TZP ceramic surface (asterisks). A more irregular and porous surface, with several gaps (arrows), was demonstrated by the Y-TZP ceramic vitrified with a ceramic liner.

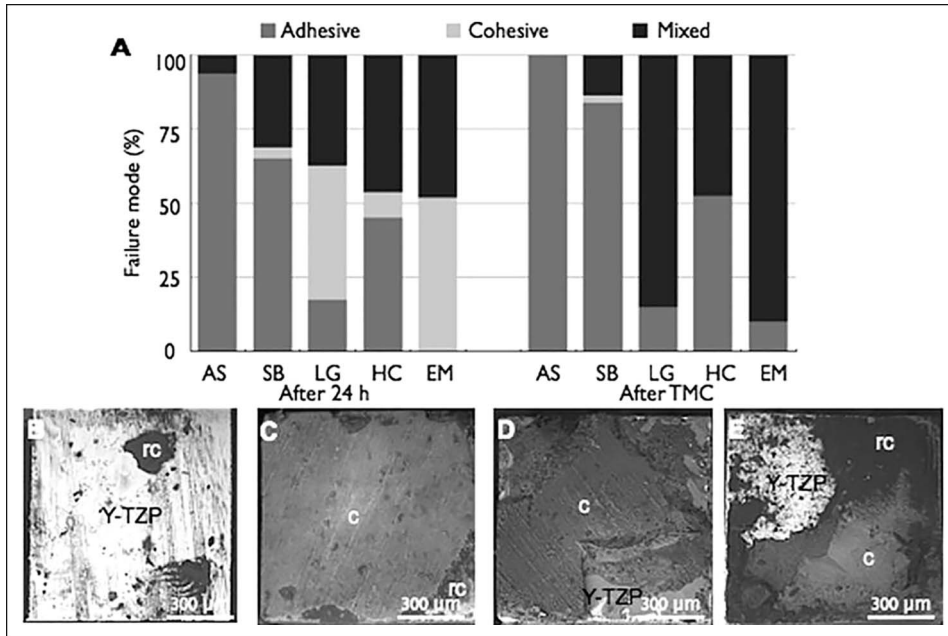


Figure 2. (A): Failure mode distribution and representative SEM images of failed beams. (B): Adhesive failure in an HC specimen. (C): Cohesive failure in an EM specimen. (D): Mixed failure in an LG specimen. (E): Mixed failure in an SB specimen. c, composite; rc, resin cement.

Representative 4D images of EM, LG, and HC surfaces before (A, B, and C) and after hydrofluoric acid etching (A', B', and C') are depicted in Figure 4. After acid etching, EM (A') presented deep craters, whereas LG (B') presented several peaks, characterizing a deep and homogeneous effect of the hydrofluoric acid. On the other hand, HC showed a more irregular topography, with areas suggesting a deeper action of hydrofluoric acid along with areas that were poorly modified by this acid.

DISCUSSION

Among other aspects, the establishment of a stable bond between a resin cement and the ceramic surface is crucial for achieving clinical longevity of any kind of Y-TZP ceramic restoration. The principal goal of the present study was to test the hypothesis that bonding of a self-adhesive resin cement to vitrified Y-TZP ceramic (LG and HC groups) could withstand TMC. The other tested groups were included for specific reasons: as-sintered Y-TZP ceramic (AS) was used as a negative control because the literature clearly demonstrates that bonding to this substrate is extremely poor.^{9,25} Contrarily, sandblasting (SB) was chosen as a positive control because this is one of the most reliable clinical protocols for preparing Y-TZP ceramic restorations for cementation.^{4,32} IPS e.max CAD (EM), a vitreous ceramic susceptible to hydrofluoric acid etching, was used to produce results intended to support the discussion about those obtained from the vitrified Y-TZP ceramic. The parameters used to perform the

TMC in the present study were chosen to simulate approximately one year of clinical service.^{33,34}

The LG group maintained the bond strength stability after TMC. On the other hand, this did not occur with the HC group. Therefore, the tested hypothesis of the present study was partially accepted. The mechanism involved in the Y-TZP ceramic vitrification processes used here could be described through the melting of the components (SiO_2 , Al_2O_3 , K_2O , Na_2O , CeO_2) present in both the glaze and liner, which were bonded to each other, creating a vitreous and Si-rich layer, capable of interacting with the Y-TZP ceramic surface through electrostatic and van der Waals forces.²¹ As a result, these vitrified Y-TZP ceramic surfaces were susceptible to hydrofluoric acid etching, thereby favoring the micro-retention of the resin cement,²⁰ a typical behavior of vitreous ceramics.

After 24 hours of water storage, AS presented the worst μTBS (Table 2), reinforcing the difficulty of establishing a suitable interaction with the nonreactive Y-TZP ceramic surface, even when using self-adhesive resin cements.⁹ Alternatively, the three treatments tested here increased the immediate bond strength to Y-TZP ceramic (Table 2). In percentage terms, this increase was 154.9% for SB, 330.9% for LG, and 223.9% for HC. Moreover, LG was the only group that presented μTBS (30.6 MPa) statistically similar to that of EM (37.3 MPa). This result suggests that vitrification using a low-fusing glaze could be a good alternative to traditional

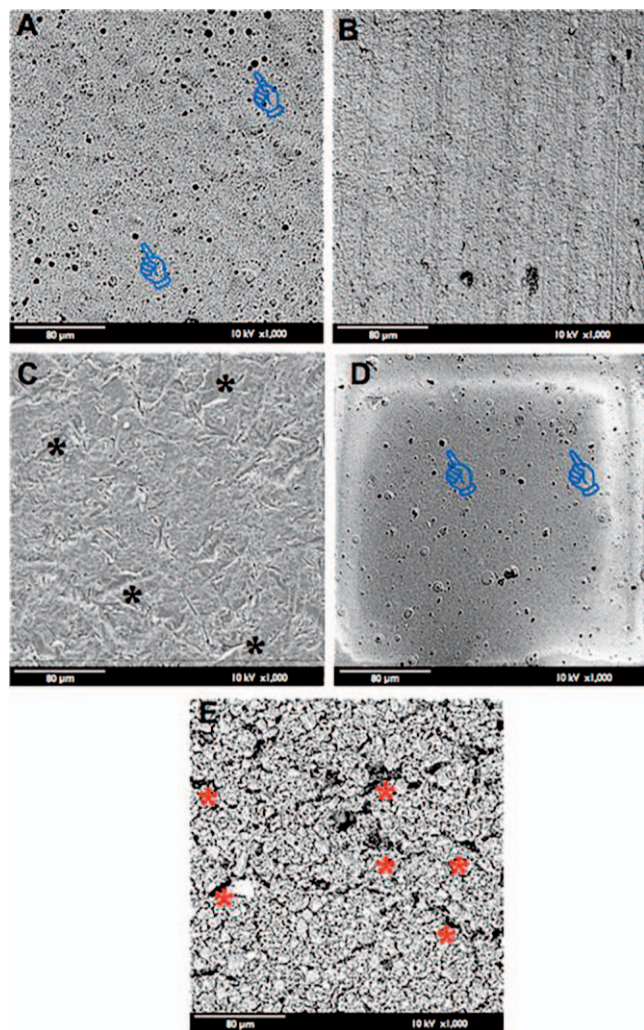


Figure 3. (A): Representative SEM images of IPS e.max CAD etched with 10% hydrofluoric acid and Y-TZP ceramic surfaces submitted to the treatments: (B) as-sintered, no surface treatment; (C) sandblasted with 50- μ m Al_2O_3 particles for 20 seconds; (D) vitrified with low-fusing ceramic glaze and etched with 10% hydrofluoric acid; and (E) vitrified with a ceramic liner and etched with 10% hydrofluoric acid for one minute.

sandblasting for improving the bond strength of resin cements to Y-TZP ceramics.

In fact, previous studies have already shown that Y-TZP ceramics vitrified with low-fusing glazes presented stronger and more stable bond strengths than when submitted to traditional protocols (sandblasting or tribochemical coating). After vitrification with two layers of a low-fusing glaze and etching with hydrofluoric acid, Cheung and others²⁵ showed that a Y-TZP ceramic presented bond strength stability after 3 weeks of water storage and thermocycling ($6000\times/5^\circ\text{C}$ - 55°C), behavior that was not presented by the groups sandblasted with Al_2O_3 particles or tribochemically coated with Si-coated

Al_2O_3 particles. Also, after thermocycling ($1800\times/5^\circ\text{C}$ and 55°C), Everson and others²⁶ showed that a Y-TZP ceramic treated with five different low-fusing glazes and hydrofluoric acid etching presented a significantly higher and more stable bond strength when compared with the tribochemically coated group. Thus, when considering that the resin cement–Y-TZP ceramic interfaces in the current study were submitted to mechanical and thermal cycling, it is reasonable to consider the results obtained here as relevant, since they show the performance of the tested bonding protocols in a worst-case scenario.³⁵

Although the LG group maintained bond stability after TMC, it still presented significantly lower μTBS than the EM group (Table 2). Figure 3 (A and D) demonstrates that both surfaces present typical aspects of vitreous ceramics etched with hydrofluoric acid.^{23,26} However, the 3D images (Figure 4) present remarkable differences in the action of hydrofluoric acid in these groups; while the EM specimen presented several craters (Figure 4A'), these structures were less visible on the LG surface, which presented picks protruding from it (Figure 4B'). Thus, it is possible that more effective micro-mechanical interlocking with the self-adhesive resin cement took place in the EM group due to the presence of these craters, thereby creating a better interaction between both materials. Even though the relationship between *in vitro* results and the clinical performance of adhesive protocols is questionable, as evidenced by the values of bond strength presented in previous studies,^{7,9,19,29} it is reasonable to consider the bond strength presented by the LG group after TMC (24.1 MPa) as indicative of good performance for indirect Y-TZP ceramic restorations submitted to this luting protocol.

Although the immediate μTBS of HC was statistically higher than that obtained for the as-sintered Y-TZP ceramic surfaces and similar to that of SB, the traditional gold standard, the μTBS of HC was lower than that of LG (Table 2). As both VITA Akzent Plus and HeraCeram Zirkonia are vitreous materials with similar composition (Table 1), this finding was somewhat surprising. However, the features depicted in Figures 3 and 4 show some possibilities for explaining this result. From the comparison of Figures 3D and 3E, the surface vitrified with HeraCeram Zirkonia (HC group) is discontinuous, with rougher grains, and presents several gaps, which are not present on the surface vitrified with VITA Akzent Plus. In addition, the Figure 4C' clearly shows that the HC surface is more

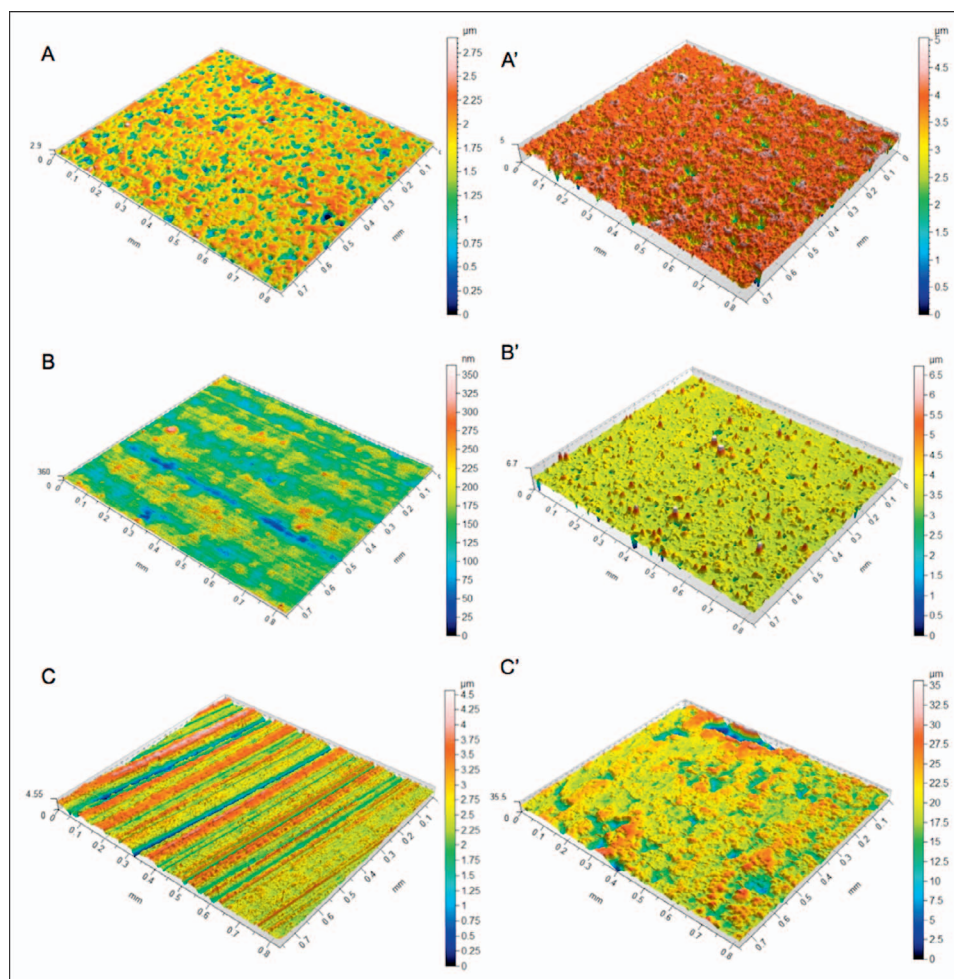


Figure 4. Representative 3D images of EM, LG, and HC surfaces before (A, B, and C) and after (A', B', and C') hydrofluoric acid etching.

irregular than the LG surface (Figure 4B'), with areas suggesting the absence of surface vitrification. Thus, one can suppose that the pattern produced by the brushing with HeraCeram Zirkonia (Figure 4C) could have favored the penetration of hydrofluoric acid through its vitreous layer, which could have partially disrupted the interaction between the liner and the Y-TZP ceramic, thereby weakening the adhesive interface. The fact that this group did not maintain bonding stability suggests that this effect was amplified after TMC.

The results observed for the SB are in agreement with earlier studies.^{36,37} The immediate μ TBS for SB was 154.9% higher than that obtained for the as-sintered surfaces, which can be explained by the increase in the micromechanical interlocking between the self-adhesive resin cement and the grooves produced by air abrasion (Figure 3C) and by the chemical interaction between the PO_4 groups present in the self-adhesive resin cement and the hydroxyl groups produced by air abrasion itself.⁸ On

the other hand, the μ TBS of the SB suffered a significant drop of 32% after TMC (Table 2). Although we did not find any previous studies that used a similar experimental protocol, other studies that evaluated air abrasion and resin cements containing phosphoric acid ester methacrylate monomers could be used to support this finding. When using the MDP-containing Panavia, Broomick and others⁴ and Quaas and others³⁸ observed a reduction in tensile bond strength from 31.86 MPa to 4.93 MPa (84%) and from 23.6 MPa to 12.1 MPa (51%), respectively, after 150 days of water storage and 37,500 thermocycles. Moreover, when submitting specimens to fatigue, Mirmohammadi and others³⁹ found a reduction from 43.9 MPa to 22.0 MPa (50%) for the flexural bond strength of Panavia bonded to a Y-TZP ceramic. In agreement with the present study, the analysis of all these results suggest that air abrasion may not be the most effective protocol for creating reliable bonding to Y-TZP ceramics.

In addition to the values of μ TBS itself, the analysis of the number of premature debonded specimens also provides important information regarding the behavior of resin cement-ceramic interfaces. It is well known that during TMC, water diffusion, thermal transfer, and the stress generated at the adhesive interfaces may accelerate the hydrolytic degradation of the adhesive polymer structures, thereby decreasing the bonding of resin cements to Y-TZP ceramics.^{25,40,41} In the current study, all of the resin composite-Y-TZP ceramic blocks from AS did not survive mechanical cycling. This finding is noteworthy because it means that the chemical interaction produced by the self-adhesive resin cement may not be strong enough to resist the loading generated during chewing. In addition, after 24 hours of water storage, 80% of the beams from AS prematurely failed during the μ TBs test (Table 3), while those that were tested presented a predominance (93.75%) of adhesive failures (Figure 2A). These aspects reflect the lowest μ TBS found for this group and again reinforce the poor chemical interaction between the phosphate groups present in self-adhesive resin cements and the passive ZrO_2 layer on the Y-TZP ceramic surface.⁹

The specimens from SB also presented a great number of premature failures, 49% and 40%, after 24 hours and TMC, respectively. This finding fits well with its intermediary values of μ TBS. A similar behavior was observed for HC. Contrarily, LG behaved similarly to EM, with only 16% and 0% of premature failures after 24 hours and TMC, respectively. Undoubtedly, this finding, associated with the maintenance of the bond strength stability observed in this group, allows advocating for vitrification with low-fusing glaze as a suitable protocol to luting Y-TZP restorations. Of course, this assumption falls within the limits of the present investigation. Furthermore, previous studies have shown that the impact of Al_2O_3 particles during sandblasting may produce subcritical microcracks and t-m phase transformation in Y-TZP ceramics, which may affect their mechanical properties.⁴²⁻⁴⁴ This is another factor defending the vitrification of Y-TZP ceramics as a more suitable protocol for improving the bond strength to this class of ceramic material.

Although the present study adds new information regarding the bonding stability to vitrified Y-TZP ceramics, it still presents some limitations, such as the use of only one resin cement and Y-TZP ceramic, as well as the absence of the impact of this approach on the dentin-resin cement adhesive interface. Moreover, the development of clinical trials could

provide evidence for considering the present outcome more clinically relevant. These aspects should be addressed in future investigations.

CONCLUSION

Within the limitations of the present investigation, the vitrification of a Y-TZP ceramic surface with a low-fusing glaze, followed by hydrofluoric acid etching, allowed the adhesive interface to better withstand TMC. In addition, this protocol showed better results than the traditional sandblasting and similar results to that observed with an IPS e.max CAD vitreous ceramic etched with hydrofluoric acid. Thus, it can be concluded that this protocol might be useful for improving the adhesion between Y-TZP ceramics and self-adhesive resin cements.

Conflict of Interest

The authors certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company presented in this article.

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Prospective Randomized Clinical Trial on the Survival of Lithium Disilicate Posterior Partial Crowns Bonded Using Immediate or Delayed Dentin Sealing: Short-term Results on Tooth Sensitivity and Patient Satisfaction

C van den Breemer • MMM Gresnigt • M Özcan • W Kerdijk • MS Cune

Clinical Relevance: No tooth sensitivity change is detected with the application of partial ceramic indirect restorations. This clinical study could not confirm that immediate dentin sealing is more advantageous than delayed dentin sealing in terms of tooth sensitivity or patient satisfaction with 1 year of clinical service of partial ceramic restorations.

<https://doi.org/10.2341/18-047-C>

Hydrolytic and Biological Degradation of Bulk-fill and Self-adhering Resin Composites

OFF de Brito • ILM de Oliveira • GQM Monteiro

Clinical Relevance: The interaction between different substances on resin composite surfaces is a critical issue in the assessment of the clinical longevity of restorative materials.

<https://doi.org/10.2341/17-390-L>

An In Vitro Investigation of Anticaries Efficacy of Fluoride Varnishes

L Al Dehailan • EA Martinez-Mier • GJ Eckert • F Lippert

Clinical Relevance: This study provides clinicians with insight into the in vitro differences in the performance of commercially available fluoride varnishes. More clinical research is needed to equip dentists with evidence that will help them make better treatment choices.

<https://doi.org/10.2341/18-040-L>

Mechanical Behavior of Ceramic Monolithic Systems With Different Thicknesses

D Longhini • COM Rocha • LT de Oliveira • NG Olenski • EA Bonfante • GL Adabo

Clinical Relevance: Highly translucent fully stabilized zirconia can be less fracture resistant than lithium disilicate and partially stabilized zirconia depending on the thickness.

<https://doi.org/10.2341/18-083-L>

Effect of Thermocycling on Biaxial Flexural Strength of CAD/CAM, Bulk Fill, and Conventional Resin Composite Materials

**EB Benalcázar Jalkh • CM Machado • M Gianinni • I Beltramini
MMT Piza • PG Coelho • R Hirata • EA Bonfante**

Clinical Relevance: Biaxial flexural strength of tested materials vary with composition and thermocycling significantly decreases their strength. Future clinical studies should evaluate if recommendations based on cavity size and tooth location improve long-term performance.

<https://doi.org/10.2341/18-146-L>

Prospective Randomized Clinical Trial on the Survival of Lithium Disilicate Posterior Partial Crowns Bonded Using Immediate or Delayed Dentin Sealing: Short-term Results on Tooth Sensitivity and Patient Satisfaction

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Clinical Relevance

No tooth sensitivity change is detected with the application of partial ceramic indirect restorations. This clinical study could not confirm that immediate dentin sealing is more advantageous than delayed dentin sealing in terms of tooth sensitivity or patient satisfaction with 1 year of clinical service of partial ceramic restorations.

SUMMARY

This prospective randomized clinical trial evaluated tooth sensitivity and patient satisfaction

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faction after the provision of partial ceramic restorations bonded using immediate (IDS) or delayed dentin sealing (DDS) on vital molar teeth through a within-subject comparison study. Between December 2013 and May 2016, a total of 30 patients (13 women, 17 men; mean age, 54 years old) received two lithium disilicate ceramic (IPS-e.max press, Ivoclar Viva-

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dent) partial restorations on vital first or second molar teeth (N=60). The two teeth randomly received either IDS (test group, n=30) or DDS (control group, n=30). Partial preparations were performed on all teeth and directly after tooth preparation. IDS was achieved using self-etch adhesive (Clearfil SE Primer and Adhesive, Kuraray) followed by the application of flowable resin (Clearfil Majesty Flow, Kuraray). Partial ceramic restorations were bonded (Variolink Ultra, Ivoclar Vivadent) two weeks after preparation. The teeth were evaluated preoperatively and at one week, three months, and 12 months postoperatively using a cold test and a questionnaire for perceived tooth sensitivity. Patient satisfaction was evaluated using a visual analog scale (VAS). Data were analyzed using McNemar, chi-squared, and Wilcoxon signed rank tests ($\alpha=0.01$). There was no significant difference in patient-reported tooth sensitivity between the preoperative phase and all other time points ($p>0.01$). There was also no significant difference between IDS and DDS ($p>0.01$) for all items on the questionnaire. VAS scores did not differ significantly between the IDS and DDS groups for all items in the questionnaire at all time points ($p>0.01$). No tooth sensitivity change was noticed with the application of partial ceramic indirect restorations. This clinical study could not confirm that IDS is more advantageous than DDS in terms of tooth sensitivity and patient satisfaction at 1 year of clinical service of partial ceramic restorations.

INTRODUCTION

Because of the advances in adhesive technologies and ceramic materials, the dental profession has positively embraced ceramic indirect restorations, as they require minimally invasive preparations. Today, biomechanically or esthetically compromised teeth can be restored with partial indirect restorations at a minimum biological price, saving sound tooth tissues. While a circumferential full crown preparation is associated with the sacrifice of 67.5% to 75.6% of the original tooth structure, partial preparation is associated with substantially less (5.5% to 27.2%).¹ The amount of tooth structure reduction could also decrease the degree of postoperative pain.¹ According to histological studies, where a distance of 0.5 mm from the pulp could cause pulpal reaction in 60% of the cases, a similar

reaction was observed in only 5% of cases in which the dentin thickness was greater than 1 mm.² Moreover, not only the preparation depth but also the provisional phase could affect pulp reaction, namely, within one or two weeks, microorganisms could reach the pulp when the dentin is not adequately sealed with a provisional restoration. Therefore, bacterial infection in the dentin tubules was postulated to be one of the main causes of pulpal damage and pain.³ To reduce the risk of postpreparation sensitivity and irritation to pulp tissue, occluding or sealing of exposed dentin tubules may be necessary. A combination product (GLUMA Desensitizer, Heraeus Kulzer GmbH, Wehreim, Germany) with glutaraldehyde and hydroxyethyl methacrylate content resulted in a significant reduction in postpreparation sensitivity.⁴ Another cause of postoperative pain is postcementation hypersensitivity. This symptom is characterized by a short, sharp pain when thermal or chemical stimuli are introduced to vital abutment teeth after the cementation of a crown or fixed dental prosthesis.⁵ This type of hypersensitivity is usually self-healing but is uncomfortable and can persist for up to 24 months.⁶ The underlying cause of dentinal hypersensitivity is in part explained by the hydrodynamic theory in that a stimulus causes fluid to flow in the dentinal tubules, and this transmits a variety of physical stimuli, which eventually stimulates electrical nerve activity, resulting in a typical, sharp, transient pain.⁷

The clinical success of ceramic restorations relies substantially on the quality of their adhesion to dental tissues, where adhesion to dentin in particular remains a clinical challenge to date. Improvements have been made over the years in terms of material properties, and more effective methods have been developed to condition intaglio surfaces of ceramic substrates. In an attempt to improve the adhesion of indirect restorations to dentin and thereby diminish postoperative sensitivity, the immediate dentin sealing (IDS) technique has been suggested as an alternative to conventional adhesive luting, also referred to as delayed dentin sealing (DDS).⁸⁻¹⁴ The major difference between the IDS and DDS technique lies in the fact that with the IDS, a thin layer of adhesive resin is applied immediately after tooth preparation and prior to impression making, whereas in the DDS, the adhesive resin layer is applied just before luting the restoration. With the IDS technique, improved patient comfort during the provisional phase, less need of anesthesia during luting of the restoration, and reduced

postoperative sensitivity were reported.^{15,16} However, IDS is an additional step in the workflow of indirect restorations, and the stability of IDS after cleansing procedures is controversial.¹⁵ A recent systematic review study emphasized the lack of randomized controlled trials and affirmed the beneficial effect of IDS to minimize tooth sensitivity.¹⁵

Therefore, the objectives of this study were to evaluate tooth sensitivity (objective and subjective) and patient satisfaction after bonding partial ceramic restorations with IDS or DDS techniques on the vital molar teeth, in a prospective, randomized within-subject clinical trial. The tested null hypothesis was that there would be no significant difference in tooth sensitivity or patient satisfaction with partial indirect restorations bonded with either IDS or DDS during the first year of clinical service.

METHODS AND MATERIALS

Study Design and Inclusion Criteria

Between December 2013 and May 2016, a total of 30 patients (13 women, 17 men; mean age: 54 years) with an indication for two indirect partial ceramic restorations for first or second vital molar teeth were recruited. Included teeth would typically be mechanically compromised because of recurrent caries, fractured cusps, old restorations with marginal leakage, and/or large restorations that would be difficult to restore with composite. Further inclusion criteria were the following: physically and psychologically able to tolerate conventional restorative procedures, good oral hygiene, presence of an intact buccal wall of the tooth, normal response on cold test, possible rubber dam application, presence of the antagonistic tooth, and willingness of the patient to return for follow-up examinations. Radiographs were taken preoperatively to verify pulpal/periapical and coronal tooth status.

The two teeth randomly received either IDS (test group, $n=30$) or DDS (control group, $n=30$) through the randomization software (www.randomizer.org). Hence, the study can be characterized as a randomized controlled, single-blind clinical trial with within-subject comparison. A consort flow chart showing the enrollment, intervention allocation, follow-up, and data analysis is presented in Figure 1. The study was approved by the Medical Ethics Committee of the University Medical Center Groningen, the Netherlands (ABR number: NL 45130), and all the patients were provided with informed consent. Distribution of restorations and extension of the restorations are presented in Table 1.

Tooth Preparation

The brands, types, manufacturers, chemical compositions, and batch numbers of the main materials used in this study are listed in Table 2. After isolating the teeth with a rubber dam (Hygienic Dental Dam, Coltène/Whaledent Inc, OH, USA), the existing restorations were removed. This step was a major determinant with respect to the preparation design, for which as much sound tooth structure as possible was maintained within the recommendations of the manufacturer for the ceramic material to be used for onlays and inlays (IPS-e.max press, Ivoclar Vivadent, Schaan, Liechtenstein). The outline configuration was a butt shoulder, prepared with a diamond bur and ultrasonic handpiece (SONICflex prep ceram, KaVo GmbH, Biberach/Riss, Germany). All internal angles were smoothed to reduce stress concentration. A minimal thickness of 1 mm for the restorative material was allowed in all areas. The cusps were covered (1.5 mm) if the remaining tooth structure wall was less than 2-mm thick from its occlusal aspect or when the outline of the restoration would be in an area with static or dynamic antagonist contacts. Slight divergence with an angle of 100° to 120° between the proximal cavity walls and the prospective proximal inlay surfaces was provided. The dental technician blocked out any incidental undercuts in the teeth that were allocated to the control group (DDS); the remaining cases were compensated for by the IDS. Contacts at the marginal ridge were avoided.

The teeth of the test group received IDS (Clearfil SE Primer and Adhesive, Clearfil Majesty Flow, Kuraray) immediately after preparation (Table 3). Electrosurgery was performed in cases in which retraction of the gingiva was required for proper impression making. Impressions were made using a silicone impression material (Heavy and Ultra Light Body Aquasil, Dentsply, Milford, DE, USA) using an individually designed impression tray. Temporary restorations were then made chairside using a chemically polymerized resin composite material (Protemp, 3M ESPE, Neuss, Germany) and cemented using polycarboxylate cement (Durelon, 3M ESPE, St Paul, MN, USA). Any temporary restorations that were debonded before the second appointment were noted.

Cementation

One dental technician fabricated all lithium disilicate restorations (IPS-e.max press, Ivoclar Vivadent). Two weeks after preparation, the temporary cement was removed from the teeth with an

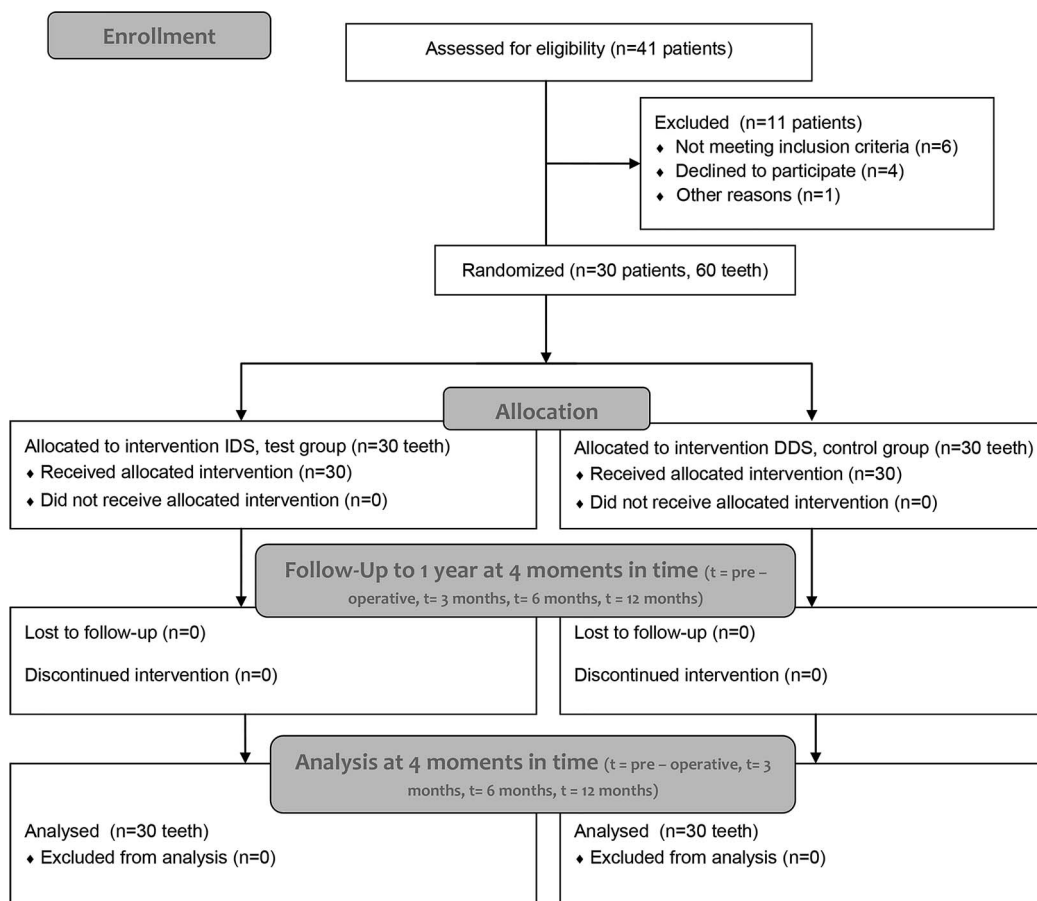


Figure 1. Consolidated Standards of Reporting Trials (CONSORT) 2010 flow diagram explaining enrollment, intervention allocation, follow-up, and data analysis.²¹

ultrasonic tip and a scaler. The IDS was checked for stability after cleansing of the preparation using magnification, but no detrimental effects were observed in any of the cases. The sequence of the different tooth-conditioning and restoration procedures, before cementation, is presented in Tables 3 and 4. The adhesive procedure differed between the test and control group, as outlined in these tables. All partial restorations were luted using a heated (55°C) dual-polymerized luting composite (Variolink Ultra, Ivoclar Vivadent). Restorations were placed initially under slight pressure, where the excess material was removed immediately from the margins with a probe, a scaler, and dental floss (Oral-B, Rotterdam, the Netherlands). After increasing the pressure, the final excess cement was manipulated against the tooth to prevent marginal gaps. The restorations were photo-polymerized (>1,000 mW/cm², Bluephase Style, Ivoclar Vivadent) for 40 seconds from three sides, and this was repeated after the application of glycerin gel. Occlusion and articulation were checked carefully using 40-µm

carbon paper. The margins of the restorations were finished using a scaler and an ultrasonic device (EVA handpiece 7LP in combination with a 61 LG, KaVo GmbH) and polished using ceramic polishers (CeraGloss blue and yellow, Edenta, Argau, Switzerland). An intraoral radiograph was then made to check for excess cement in the cervico-approximal region. A typical case is presented in Figure 2.

Evaluation

The test and control teeth were initially dried with gauze. Subsequently, objective tooth sensitivity was measured using a coolant spray (Kälte spray, Orbis Dental, Münster, Germany) on a cotton ball (4 mm) that was immediately applied to the buccal wall of the corresponding tooth in each group, and the time until the patient responded was recorded. Regarding subjective tooth sensitivity, the patients were asked to indicate which one of the two teeth was the most sensitive or whether they were equally sensitive upon cold testing, when drinking hot/cold beverages, or while chewing. The patients were also asked to

Table 1: *Distribution of Restored Teeth and Extension of the Restorations in the Maxilla and Mandible in the Test (Immediate Dentin Sealing; IDS) and Control (Delayed Dentin Sealing; DDS) Groups*

	Molars, n					Total, N
	Zero Cusps Replaced	One Cusp Replaced	Two Cusps Replaced	Three Cusps Replaced	Four Cusps Replaced	
Test group						
Maxilla, n	2	4	5	2	6	19
Mandible, n	2	1	1	4	3	11
Total, N	4	5	6	6	9	30
Control group						
Maxilla, n	5	1	1	2	5	14
Mandible, n	2	3	5	2	4	16
Total, N	7	4	6	4	9	30

Table 2: *Brands, Types, Manufacturers, Chemical Compositions, and Batch Numbers of the Major Materials Used in This Study*

Brand	Type	Manufacturer	Chemical Composition	Batch-Number
IPS-e.max Press	Pressable ceramic	Ivoclar Vivadent, Schaan, Liechtenstein	SiO ₂ , LiO, K ₂ O, P ₂ O ₅ , ZrO ₂ , ZnO	—
Variolink Ultra Catalyst/Base	Dual-cure luting composite	Ivoclar Vivadent	Ytterbium trifluoride, Bis-GMA, urethane dimethacrylate, triethylene glycol dimethacrylate, dibenzoyl peroxide, titanium dioxide	S27220/S06644
Clearfil SE Primer	Adhesive primer	Kuraray Co Ltd, Osaka, Japan	HEMA, hydrophilic dimethacrylate, water, photo-initiator	2U0022
Clearfil SE Bond	Adhesive resin	Kuraray Co Ltd	MDP, HEMA, bis-GMA, hydrophilic dimethacrylate, water photo initiator, silanated colloidal silica	2T003
Excite F DSC	Single component adhesive	Ivoclar Vivadent	Phosphonic acid acrylate, dimethacrylates, hydroxyethyl methacrylate, highly dispersed silicon dioxide, ethanol catalysts stabilizers, fluoride	S36470
CoJet-sand	Blast-coating agent	3M ESPE, St Paul, MN, USA	Aluminium trioxide particles coated with silica, 30 µm	446317 534151/
ESPE-Sil	Silane coupling agent	3M ESPE	Ethyl alcohol, 3-methacryloxypropyl-trimethoxysilane ethanol	518272
Monobond Plus	Silane coupling agent	Ivoclar Vivadent	Ethanol, 3-trimethoxysilpropyl-methacrylate methacrylated phosphoric acid ester	S31153
IPS Ceramic etch	Hydrofluoric acid	Ivoclar Vivadent	<5% hydrofluoric acid	S26140
IPS Neutralizing powder	Neutralizing powder	Ivoclar Vivadent	Sodium Bicarbonate	S34285
Ultra-etch	35% Phosphoric acid	Ultradent, South Jordan, UT, USA	35% phosphoric acid	130910
Clearfil Majesty Flow	Photopolymerizing composite	Kuraray Co	Triethylene glycol dimethacrylate, hydrophobic aromatic dimethacrylate, silanated barium glass filler, silanated silica filler, dl-camphorquinone	00339BA
Glycerin gel	K-Y* lubricating gel	K-Y, Johnson & Johnson, Sezanne, France	Purified water, glycerin, methylparaben, propylparaben, propylene glycol, hydroxyethylcellulose, disodium, phosphate, sodium phosphate, tetrasodium, EDTA	1233V

Table 3: Clinical Protocol for the Test Group (Immediate Dentin Sealing; IDS) and control Group (Delayed Dentin Sealing; DDS)

Working Time, s	
Test group	
Visit 1: Tooth preparation	
1.1 Tooth preparation	
1.2 Apply Clearfil SE Primer, active brushing motion	20
1.3 Air suction	
1.4 Apply Clearfil SE Adhesive, active brushing motion	10
1.5 Air thin	10
1.6 Photo polymerize	10
1.7 Apply flowable resin (Clearfil Majesty flow)	
1.8 Photo polymerize	40
1.9 Apply glycerin gel	
1.10 Photo polymerize at buccal, oral, and proximal sites	40 each
1.11 Rinse until clean surface	
1.12 Clean the enamel outline with a rubber point or a bur	
1.13 Make impression	
Visit 2: Cleaning and conditioning of the tooth prior to luting	
2.1 Clean tooth surface (EMS)	
2.2 Silica coat (CoJet-sand) the IDS	2-3
2.3 Acid etch enamel	30
2.4 Rinse	30
2.5 Dry	
2.6 Apply silane (ESPE-sil) on the IDS	60
2.7 Apply adhesive resin (Excite F DSC)	
2.8 Apply resin cement (Variolink Ultra) on the tooth	
2.9 Place the partial restoration on the tooth	
2.10 Remove excess cement	
2.11 Photo polymerize at buccal, oral, and proximal sites	40 each
2.12 Apply glycerin gel	
2.13 Photo polymerize at buccal, oral, and proximal sites	40 each
Control group	
Visit 1: Tooth preparation	
1.1 Tooth preparation	
1.2 Make impression	
Visit 2: Cleaning and conditioning of the tooth prior to luting	
2.1 Clean tooth surface (EMS)	
2.2 Acid etch enamel / dentin	30/15
2.3 Rinse	30
2.4 Dry	
2.5 Apply adhesive resin (Excite F DSC)	
2.6 Apply resin cement (Variolink Ultra) on the tooth	
2.7 Place the partial restoration on the tooth	
2.8 Remove excess cement	
2.9 Photo polymerize at buccal, oral, and proximal sites	40 each
2.10 Apply glycerin gel	
2.11 Photo polymerize at buccal, oral, and proximal sites	40 each

Table 4: Clinical Protocol for Luting Procedures of the Ceramic Restorations	
Visit 2: Luting Procedures of the Ceramic Restorations	Working Time, s
2.1 Apply hydrofluoric acid etch (IPS ceramic etch)	20
2.2 Rinse and neutralize	60
2.3 Rinse and dry	
2.4 Ultrasonically clean in distilled water	300
2.5 Dry	
2.6 Apply silane (Monobond plus; one coat) and wait for its reaction	60
2.7 Apply adhesive resin (Excite F DSC)	
2.8 For the subsequent procedures, follow step 2.8 in Table 3 (test group) or step 2.6 in Table 3 (control group)	

score the degree of experienced discomfort after the placement of the restoration using a visual analog scale (VAS), with scores ranging from *none* to *very painful* (Figure 3). Both evaluations were performed preoperatively and repeated after one week and then three and 12 months postoperatively.

To evaluate patient satisfaction, the patients were asked how satisfied they were with the restorations in general, with regard to the color and shape of the restorations, and the ability to chew (VAS, ranging from *very satisfied* to *not satisfied*) per tooth (Figure 4). These questions were posed to the patient after one week and then three and 12 months postoperatively.

Patients were instructed to call if they experienced any kind of failure (debonding or fracture) or serious discomfort or other concerns.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software for Windows (SPSS Inc, Chicago, IL, USA). Objective tooth sensitivity was compared at all time points using the McNemar test. To compare the frequency of subjective tooth sensitivity across treatments (IDS vs DDS), a chi-square test was applied to the measurements at each time point. The VAS items regarding tooth sensitivity and patient satisfaction were first tested for normality. As none of the items were distributed normally, all sensitivity and satisfaction items of both treatments were compared using Wilcoxon signed rank tests. Temporary crown loosening was compared across treatments using McNemar test. *p* values less than 0.01 were considered to be statistically significant in all tests.

RESULTS

After 1-year follow-up, all restorations (N=60) were *in situ* and showed a normal reaction to cold testing.

None of the patients called to report failure, discomfort, or other concerns before the 1-year follow-up. No further technical or biological complications were noted. No tooth sensitivity change was noticed with partial preparations.

Tooth sensitivity reports of the patients did not show a significant difference between the preoperative phase and at all other time points (*p*>0.01; Table 5). There was also no significant difference between IDS and DDS (*p*>0.01) for all items in the questionnaire.

Patient satisfaction according to the VAS scores was not significantly different between the IDS and DDS groups for all items in the questionnaire at all time points (*p*>0.01; Table 5).

The frequency of debonding in the temporary restorations in the IDS group was higher (N=7; inlay: 1, two-cusp: 1, three-cusp: 2, four-cusp replacement: 3) than the control group (DDS; N=3, two-cusp: 1, three-cusp: 1, four-cusp: 1), but the difference in debonding rate per patient was not significantly different in both groups, $\chi^2(1, n=60)=1.125, p=0.289$.

DISCUSSION

This study was undertaken to investigate tooth sensitivity (objective and subjective) and patient satisfaction after bonding partial ceramic restorations using the IDS or DDS techniques on vital molar teeth. The key finding of this study indicated no significant difference in tooth sensitivity or patient satisfaction with partial restorations bonded with either IDS or DDS during the first year of follow-up. Thus, the null hypothesis was accepted.

The amount of tooth structure reduction is considered to be an important factor affecting postoperative tooth sensitivity.¹ One study that compared the effects of two different cements on tooth sensitivity of full circumferential crowns

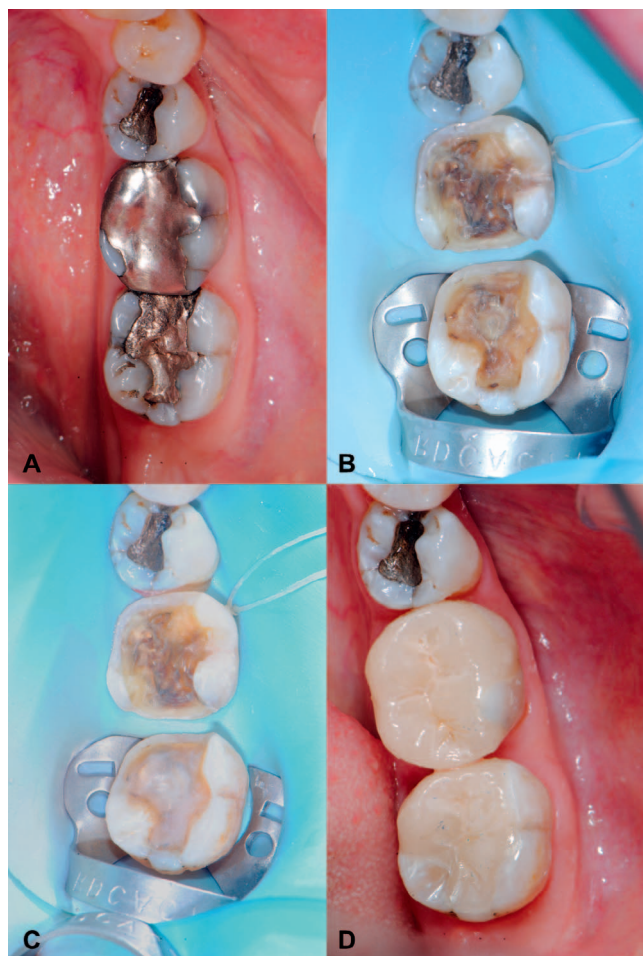


Figure 2. Typical case situation for tooth 30 and 31. (a) Initial situation. (b) Situation after removing the old restorations, before the application of IDS on tooth 31. (c) Same as with situation b, but IDS is applied on the second molar tooth. (d) Situation after luting the lithium-disilicate restorations.

concluded that postcementation tooth sensitivity was a frequent complaint.¹⁷ This is in contrast to our findings, in which none of the patients complained about postcementation sensitivity, probably because of the minimal invasive preparation design. The use of IDS was postulated to reduce postoperative tooth sensitivity, improve comfort during the provisional restoration period, and necessitate anesthesia less frequently during cementation.^{15,16} In this study, however, the added value of IDS on perceived tooth sensitivity was not demonstrated using a minimally invasive preparation approach. This is also in contrast with the findings of Hu and Zhu,¹⁶ who found that the IDS technique significantly reduced postcementation tooth sensitivity in abutment teeth restored with fixed dental prostheses. In that study, full circumferential crown preparations were performed. More postoperative

sensitivity may be expected in full circumferential preparations as more dentin tubules are opened on the surface and these are intimately connected to the pulp. Also, Hu and Zhu¹⁶ used a bonding system with a separate etching step. Self-etching systems have a tendency to minimize postoperative sensitivity, compared with total-etch or milder etching systems, because of their higher qualitative and quantitative capacity of penetration.¹⁸ When the risk of postoperative tooth sensitivity is expected to be high, such as in deep preparations or with the use of total-etch systems, the addition of IDS may make a difference in reducing postoperative sensitivity.

In this study, normal response was noted upon provocation with a cold test after drying the teeth with gauze followed by a question about the difference in sensitivity between the teeth as an outcome variable for perceived sensitivity. Cold tests have been proved to be more reliable than heat tests in determining pulp vitality, but their accuracy is certainly not absolute.¹⁹

All test and control group evaluations on satisfaction were comparable. In fact, when people within the control group were already very satisfied, then it is difficult to demonstrate any relevant increase in the test group. Also, satisfaction was strongly related to communication, approach, and involvement and not always directly related with the clinical outcome. Moreover, not all satisfaction items were relevant when comparing differences between the test and control group.

A meta-analysis related to the topic indicated restoration failures in the first year predominantly due to endodontic complications.²⁰ The results of this study after 1 year were promising since no endodontic complications occurred within the first year. Teeth from the test group had a tendency to debond more often in the temporary crown phase than teeth in the control group. This did not influence the difference in patient-perceived tooth sensitivity between the two teeth, but it is certainly not desirable in a clinical setting. The temporary cement in this study was polycarboxylate cement, which reacts chemically with the calcium ions in the tooth structure. This may explain why the cement attached better to the tooth in the control group. In the test group, however, the dentin was covered with adhesive from the IDS layer. In such a situation, the cement cannot adhere adequately to the tooth and may yield to debonding. Our null hypotheses that there would be no significant difference in tooth sensitivity and patient satisfaction with partial indirect restorations bonded using IDS or DDS

Fig 3

1. This tooth hurts when drinking warm/cold beverages.

Tooth 1: yes / no (circle the correct answer)

Tooth 2: yes / no (circle the correct answer)

2. This tooth hurts when chewing.

Tooth 1: yes / no (circle the correct answer)

Tooth 2: yes / no (circle the correct answer)

3. I experienced pain after the restoration was placed
(not registered at t=pre-operative)

Tooth 1: None |-----|-----| Very Painful
0 10

Tooth 2: None |-----|-----| Very Painful
0 10

(To be filled in after cold testing)

4. This tooth is more painful (cross the correct answer)
(t=pre-operative, t=1 week, t=3 months, t=12 months)

Tooth 1 ☐

Tooth 2 ☐

No difference between teeth ☐

Figure 3. Questionnaire assessing perceived tooth sensitivity.

Figure 4. Questionnaire used for the assessment of patient satisfaction.

Fig 4

1. Can you indicate how satisfied you are with the crowns in general?

2. Can you indicate how satisfied you are with the colour of the crowns?

3. Can you indicate how satisfied you are with the form of the crowns?

4. Can you indicate how satisfied you are with being able to chew with the crowns?

Answers following this answer example:

Tooth 1: Very Satisfied |-----|-----| Not Satisfied
0 10

Tooth 2: Very Satisfied |-----|-----| Not Satisfied
0 10

during the first year of clinical service were not rejected.

CONCLUSIONS

From this clinical study, the following can be concluded:

- 1. No tooth sensitivity change could be detected with a minimally invasive preparation design.

- 2. Perceived tooth sensitivity after partial preparation of vital molar teeth and their subsequent restoration with adhesively luted glass ceramic was not affected with the implementation of IDS or DDS at all follow-up moments for all items in the questionnaire.
- 3. Patient satisfaction with regard to the color and shape of the restorations and the ability to chew were not significantly different between the IDS and DDS groups at all time points.

Table 5: Results of Perceived Tooth Sensitivity and Patient Satisfaction ($\alpha=0.01$) at All Time Points (McNemar, Wilcoxon Signed Rank, and Chi-Square Tests)

	Preoperative				1 week				3 months				12 months						
Perceived tooth sensitivity	DDS>IDS	DDS=IDS	DDS<IDS	p-value	DDS>IDS	DDS=IDS	DDS<IDS	p-value		DDS>IDS	DDS=IDS	DDS<IDS	p-value		DDS>IDS	DDS=IDS	DDS<IDS	p-value	
Which tooth is most sensitive upon cold testing?	30%	30%	40%	0.74	3%	33%	30%	0.91		40%	33%	27%	0.67		27%	43%	30%	0.50	
	DDS	IDS-NO	IDS-YES	p-value	DDS	IDS-NO	IDS-YES	p-value		DDS	IDS-NO	IDS-YES	p-value		DDS	IDS-NO	IDS-YES	p-value	
This tooth is sensitive when drinking hot or cold beverages (yes/no)	No	2.9	0	1	No	2.2	2	1		No	2.6	1	1		No	3.0		x	
	Yes	1	0		Yes	2	4			Yes	1	0			Yes				
This tooth is tender while chewing (yes/no)	DDS	IDS-NO	IDS-YES	p-value	DDS	IDS-NO	IDS-YES	p-value		DDS	IDS-NO	IDS-YES	p-value		DDS	IDS-NO	IDS-YES	p-value	
	No	2.9	1	1	No	2.4	1	0.63		No	3.0		x		No	3.0		x	
I have experienced pain after placement of the restoration (VAS, Range, none whatsoever - very painful)	Yes	0	0		Yes	3	2			Yes					Yes				
					DDS>IDS	DDS=IDS	DDS<IDS	p-value	Z	DDS>IDS	DDS=IDS	DDS<IDS	p-value	Z	DDS>IDS	DDS=IDS	DDS<IDS	p-value	Z
					23%	47%	30%	0.73	-0.34	10%	63%	27%	0.34	-0.95	7%	70%	23%	0.14	-0.84
Patient Satisfaction					DDS>IDS	DDS=IDS	DDS<IDS	p-value	Z	DDS>IDS	DDS=IDS	DDS<IDS	p-value	Z	DDS>IDS	DDS=IDS	DDS<IDS	p-value	Z
General satisfaction (VAS, Range, none whatsoever - very painful)					23%	53%	23%	0.88	-0.16	17%	70%	14%	0.62	-0.49	20%	70%	10%	0.40	-0.1
Satisfaction with the color of the restoration (VAS, Range, none whatsoever - very painful)					3%	83%	13%	0.26	-1.1	14%	67%	20%	0.53	-0.6	13%	73%	13%	0.94	-0.9
Satisfaction with the shape of the restoration (VAS, Range, none whatsoever - very painful)					13%	77%	10%	0.60	-0.5	10%	60%	30%	0.05	-2	23%	63%	13%	0.35	-0.7
Satisfaction with the ability to chew with the restoration (VAS, Range, none whatsoever - very painful)					20%	50%	30%	0.31	-1	13%	73%	13%	0.89	-0.1	13%	63%	23%	0.51	-1.5

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Medical Ethics Committee of the University Medical Center Groningen. The approval code for this study is: ABR number: NL 45130.

Conflict of Interest

The authors' institutions supported this study. The authors declare that they have no conflicts of interest and that they did not have any commercial interest in any of the materials used in this study.

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Hydrolytic and Biological Degradation of Bulk-fill and Self-adhering Resin Composites

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Clinical Relevance

The interaction between different substances on resin composite surfaces is a critical issue in the assessment of the clinical longevity of restorative materials.

SUMMARY

Objectives: This study aimed to evaluate the hydrolytic degradation (*in vitro*) and biodegradation (*in situ*) of different resin composites: bulk-fill (XTra Fill, XTF/VOCO; Tetric EvoCeram Bulk Fil, TBF/ Ivoclar Vivadent), self-adhering (Vertise Flow, VTF/ Kerr; Fusio Liquid Dentin, FUS/ Pentron Clinical), and a conventional resin composite (Filtek Z250, Z250/ 3M ESPE), which was used as a control.

Methods and Materials: Seventy-five cylindrical specimens (7×1 mm) were desiccated and immersed into distilled water (DW), artificial saliva (AS), and 0.1 M lactic acid (LA) ($n=5$) for 180 days. Specimens were weighed after 180

days, after which they were desiccated again. The sorption ($\mu\text{g}/\text{mm}^3$) and solubility ($\mu\text{g}/\text{mm}^3$) were calculated based on ISO 4049. For the *in situ* phase, an intraoral palatal device containing five cylindrical specimens (5×1.5 mm) was used by 20 volunteers for seven days. Surface roughness was evaluated before and after this period to analyze the superficial biodegradation. Sorption and solubility data were submitted to Kruskal-Wallis and Mann-Whitney tests. The Wilcoxon signed-rank test was used to compare roughness at different observation times. The statistical significance for all tests was considered $\alpha=0.05$.

Results: For *in vitro*, self-adhering resin composites (VTF and FUS) showed, respectively, higher sorption values independent of the solution (62.55 and 50.81 $\mu\text{g}/\text{mm}^3$ in DW, 67.26 and 50.46 $\mu\text{g}/\text{mm}^3$ in AS, and 64.98 and 59.86 $\mu\text{g}/\text{mm}^3$ in LA). Self-adhering VTF also had a greater solubility value in DW (22.18 $\mu\text{g}/\text{mm}^3$) and FUS in LA (65.87 $\mu\text{g}/\text{mm}^3$). In AS, the bulk-fill resin composite XTF showed higher solubility (22.13 $\mu\text{g}/\text{mm}^3$). All resins were biodegraded, but the XTF specimens were more resistant ($p=0.278$) to chemical attack.

Conclusions: The self-adhering resin composites showed the highest hydrolytic degradation, and the bulk-fill resin composites exhib-

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ited comparable or superior results to the conventional resin composites. Not all resin composites underwent biodegradation in the *in situ* environment. The storage environment influenced the final characteristics of each material tested.

INTRODUCTION

The complexity of the oral environment is a significant challenge for restorative materials. The presence of water, saliva, and the acid metabolites produced by the cariogenic biofilm may lead to physical and hydrolytic degradation of the dental restorations. Moreover, surface damage such as softening and increased surface roughness can also occur as part of the biodegradation process.¹⁻³ The clinical desire for more straightforward restorative procedures leads to the development of new restorative materials. New monomer technologies, photoinitiators, or bonding mechanisms have been introduced such as bulk-fill and self-adhering resin composites. However, there is limited knowledge about the influence of cariogenic biofilms on the hydrolytic and biological degradation of these resin composites.⁴

The resistance to degradation in the oral environment has a significant impact on the longevity and clinical success of dental materials.⁵ Sorption and solubility are inherent bulk properties of the polymeric materials. Besides the deleterious effects on the structure of the material, these properties may predict material behavior and clinical performance, as they are precursors to a variety of physical and chemical processes of biological concern.^{5,6} The degree of sorption and solubility can influence the restorative's integrity, esthetic appearance, and surface properties.⁷

The sorption through polymeric materials is considered a diffusion-controlled phenomenon that is influenced by the hydrophilicity of the polymer network,⁸ degree of conversion,⁹ and cross-link density¹⁰; it is inversely proportional to the amount of filler particles.^{11,12} Sorption is a result of absorption and adsorption. Adsorption is a surface phenomenon, whereas absorption involves the whole volume of the material.¹³ Dental polymer networks based on dimethacrylate monomers absorb moisture, which may result in the enlargement of the spaces between the polymer chains.¹⁴ These areas allow free unreacted monomers trapped in the polymer network to diffuse out into the storage media, depending on their molecular size and their affinity to the solvent.¹⁵ The solvent can reduce the siloxane

bonding energy between the filler particle and the resin matrix, and with time, can break this bond and allow the solvent adsorption to the particle.¹³ Thus, the solubility can compromise the biocompatibility of the material and lead to mechanical property reduction.^{8,14}

Many immersion media have been tested such as water,^{8,16} saliva,^{17,18} lactic acid,¹⁷ and esterases,¹⁵ each with well-established protocols. The acid metabolites produced by cariogenic biofilm can cause increased surface roughness.¹⁹ *Streptococcus mutans* has higher affinity to resin composites than enamel and other restorative materials such as ceramics and metals.²⁰ Organic acids may degrade methacrylate-based polymers, changing their surface hardness.^{21,22} The biostability of resin composites impacts the prevalence of secondary caries.^{23,24}

The bulk-fill resin composites are based on new monomer technology and polymerization modulators⁹ that reduce the effect of polymerization shrinkage stress.¹⁰ The self-adhering restorative resin composites are based on traditional methacrylate systems, with the incorporation of functional acidic monomers typically found in dentin bonding agents, such as glycerolphosphate dimethacrylate (GPDM). According to the manufacturers, such monomers may be capable of bonding through mechanical and chemical interactions with the tooth structure.¹⁹

Therefore, the objective of this study was to analyze the *in vitro* hydrolytic degradation and the *in situ* biodegradation of bulk-fill and self-adhering resin composites in different immersion media generally present in the oral environment. The null hypotheses tested were: (i) there is no difference in the sorption and solubility between evaluated materials; (ii) after 180 days of storage, there is no difference in the influence of storage medium on the restorative materials tested; and (iii) biofilm interaction does not promote increase in surface roughness.

METHODS AND MATERIALS

This study was conducted in two parts: *in vitro* (Figure 1) and *in situ* (Figure 2). All tested materials are listed in Table 1.

The sample calculation was performed using BioEstat 5.3 (Mamirauá Sustainable Development Institute, Manaus, Brazil). An $\alpha = 0.05$ and a 0.80 power was considered. For the *in vitro* test, the results from the study of Alshali and others¹⁴ were considered. The ideal sample size per group was four to note

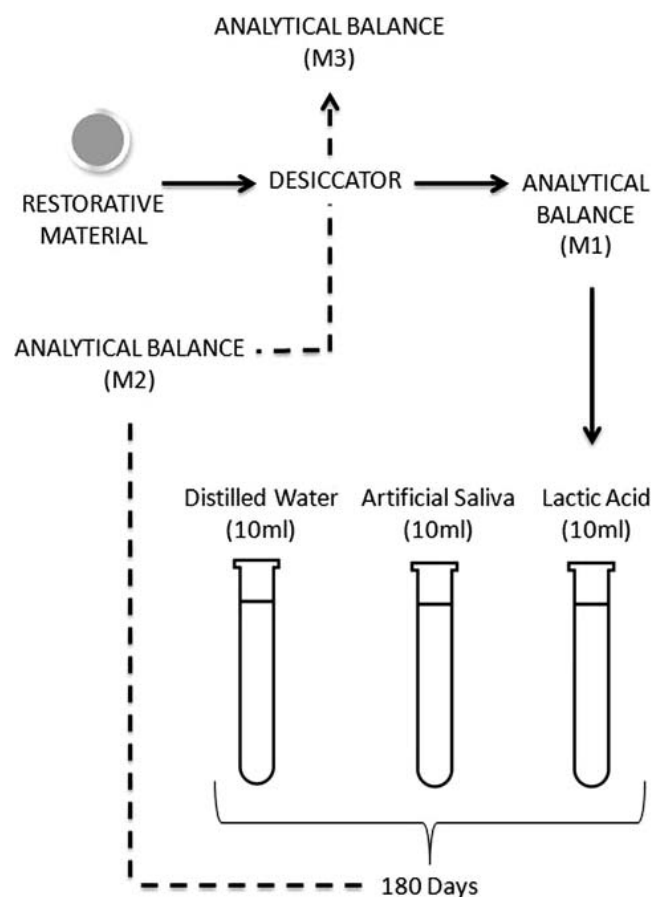


Figure 1. Schematic illustration of the in vitro experimental design.

significant differences. In this study, a sample size of five was used. For the *in situ* phase, the sample calculation was based on the study of Padovani and others.³ A sample size of 17 was found to note significant differences; however, a sample size of 20 volunteers was used to compensate possible outlying values that might lead to specimen loss.

Preparation of Specimens for the *In Vitro* Phase

Seventy-five disc-shaped specimens of each resin composite were made using a Teflon mold (7×1 mm). The mold was filled to excess and a Mylar matrix strip under a microscope glass slab were placed on top. Slight digital pressure was applied to the glass to minimize voids.

Each specimen was cured at the specimen's central area according to the manufacturers' instructions using an LED light curing unit (Radii-Cal, Dental Product SDI, Bayswater, Victoria, Australia) with 1200 mW/cm² irradiance. Specimens were removed from the mold, and the flash was removed using a sharp blade and silicon carbide papers (#600 and #1200).

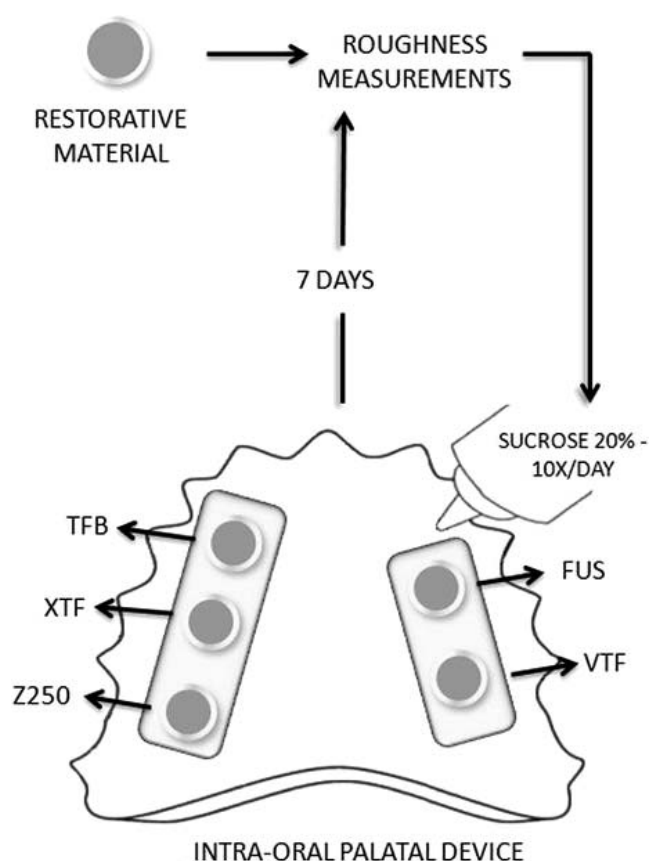


Figure 2. Schematic illustration of the in situ experimental design and the intraoral palatal device model.

Sorption and Solubility

The specimens were individually stored in plastic vials, placed in a desiccator containing freshly dried silica gel, and transferred to an incubator at 37°C. After 24 hours, the discs were repeatedly weighed on an analytical balance (AUW 220D/0.01 mg, Shimadzu Analytical Balance, Tokyo, Japan) until specimen mass variation was less than ± 0.1 mg to obtain m_1 , the initial mass of each specimen. The dimensions of the specimens were measured at four points using a digital caliper (Digimess, São Paulo, SP, Brazil) with a precision of 0.01 mm, and the volume (V) was calculated in mm³ using the mean diameter and thickness of each specimen. Specimens were divided into three groups ($n=5$) according to the immersion media used: distilled water, artificial saliva (Table 2), or lactic acid 0.1 M. The pH of lactic acid was adjusted to 4.0 with 40% sodium hydroxide (Dilecta, Compounding Pharmacy, João Pessoa, Brazil). Each specimen was placed in a plastic vial, and 10 mL of immersion medium was added and kept at $37 \pm 1^\circ\text{C}$ during all experimental phases.

Table 1: Tested Materials, Composition, and Specifications						
Materials	Classification	Monomers*	Inorganic Filler	Filler, wt/vol %	Cure Time, s	Manufacturer Batch Number
X-Tra Fil (XTF)	Bulk Fill Sculptable Resin Composite	Bis-GMA, UDMA, TEGDMA	Barium aluminum silicate vitreous particles	86/70.1	10	VOCO, Cuxhaven, Germany, 1443074
Tetric EvoCeram Bulk Fill (TBF)	Bulk Fill Sculptable Resin Composite	Bis-GMA, UDMA, Bis-EMA	Vitreous particles of barium aluminum silicate, Ytterbium trifluoride, Mixed oxide	62.5/61	10	Ivoclar Vivadent, Ontario, Canada, T32776
Vertise Flow (VTF)	Self-Adhering Flowable Resin Composite	GPDM, HEMA	Prepolymerized filler, barium glass, nano-sized colloidal silica, nano-sized ytterbium fluoride	70/NA	20	Kerr, Orange, CA, USA, 5245261
Fusio Liquid Dentin (FUS)	Self-Adhering Flowable Resin Composite	UDMA, TEGDMA, HEMA, 4-MET	Amorphous silica nanoparticulate, silane treated with barium glass, minor additives, photoactivating particles	65/52	10	Pentron Clinical, Orange, CA, USA, 5224286
Filtek Z250 (Z250)	Universal Sculptable Resin Composite	UDMA, Bis-GMA, Bis-EMA, TEGDMA	Particles of zirconia and silica	82/60	20	3M/ESPE, St Paul, MN, USA, 269759
* Bis-EMA, bisphenol A ethoxylate dimethacrylate; Bis-GMA, bisphenol A glycidyl dimethacrylate; GPDM, glycerolphosphate dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; 4-MET, 4 methacryloxyethyl trimellitic acid.						

After a 180-day period, the specimens were blotted dried with absorbent paper until moisture could no longer be observed and the recorded mass was registered as m_2 . All the specimens were then reconditioned in the desiccator and then weighed until the mass variation was smaller than 0.1 mg (m_3). The sorption (S_p) and solubility (S_l) of the resin composites (mg/mm³) were calculated according to the following equations:

$$S_p = \frac{(m_2 - m_1)}{V}$$

$$S_l = \frac{(m_1 - m_3)}{V}$$

Preparation of Specimens for *In Situ* Phase

For this phase, 100 disc-shaped specimens were made (5×1.5 mm) as previously described. However,

these specimens had their surfaces polished by using fine and superfine aluminum oxide abrasive discs (Sof-Lex 3M ESPE, St. Paul, MN, USA) for 20 seconds in the same direction with intermittent movements. The specimens were then washed in an ultrasonic cleaner (1440D, Biodont, São Paulo, SP, Brazil) with water for 30 seconds.

Roughness Measurements

The mean surface roughness (Ra) was measured (μm) using a contact profilometer (SURFTEST SJ 310, Mitutoyo Corp, Kanagawa, Japan). Four successive in-line measurements were performed in the horizontal direction of each disc at the constant speed of 0.5 mm/s with a load of 0.7 mN, and a cutoff value of 0.25 mm, to maximize filtration of surface waviness, and a trace length of 4.0 mm. Calibration was done periodically to monitor the instruments' performance.

Table 2: Chemical Composition of Artificial Saliva		
Saliva 1 Composition	Saliva 2 Composition	Saliva Final Composition
0.625% potassium chloride	10% sodium benzoate	10% of saliva 1 composition
0.865% sodium chloride	42.74% sorbitol	10% of saliva 2 composition
0.0558% magnesium chloride	1.1295% potassium acid phosphate	0.5% carboxymethyl cellulose
0.1662% calcium chloride	0.0043% sodium fluoride	0.1% phenonip
Distilled water Q.S.P 100%	Distilled water Q.S.P 100%	Distilled water Q.S.P 100%
Abbreviation: Q.S.P, French for quantité suffisante pour.		

Panelists and Ethical Aspects

This study protocol was approved by the Local Research and Ethics Committee (protocol 895.899/2014) where 20 individuals, ages 21 to 30 years and dental students in graduate and undergraduate courses, participated in this study. The inclusion criteria were as follows: good general and oral health, the absence of antibiotic use two months before the experiment, no signs of gingivitis or caries, and ability to comply with the study. Volunteers using a dental prosthesis or orthodontic devices, with diabetes, palatine torus, chronicle oral breathing, or with motor difficulties were excluded from the experiment. Visual and oral examinations were carried out by a dentist. All the volunteers agreed to participate and signed an informed written consent form.

In Situ Phase

The volunteers' teeth were impressed with alginate (Hidrogum 5, Zhermack, Rovigo, Italy), and type III gypsum models were obtained. An acrylic intraoral palatal device was made for each participant, each containing five wells (2.5 mm deep) as shown in Figure 2. A plastic mesh was fixed to the sides of the intraoral device covering each specimen. A 1.0-mm space was left between the mesh and the specimen to allow dental biofilm accumulation to form.

Oral and written instructions were given to the volunteers. No dietary restrictions were given, but participants were asked to remove the device before eating or drinking beverages and to keep the device moist in a plastic box provided. Oral hygiene was indicated three times per day with a standardized fluoride dentifrice (Colgate Maximum Cavity Protection, Colgate-Palmolive Company, New York, NY, USA). At this time, only the palatal region of the device was extraorally brushed to avoid disturbing the biofilm. The cariogenic challenge was provided by an extraoral application of a 20% sucrose solution on the specimens at 10 times per day. For this application, the devices were removed from the mouth, and the excess saliva was cleaned with gauze. One drop of the solution was placed on each specimen at 08:00, 09:30, 11:00, 12:30, 14:00, 15:30, 17:00, 18:30, 20:00, and 21:30 hours. The sucrose was then gently dried with gauze after five minutes, and the device was reinserted into the mouth. After seven days, the specimens were carefully removed from the device and washed in an ultrasonic bath for 10 minutes. The final roughness measurements were then performed as previously described.

Statistical Analysis

Data were analyzed using STATA/SE software 12.0 (StataCorp, College Station, TX, USA). Means and standard deviations were calculated. The presence of normal distributions was evaluated by the Kolmogorov-Smirnov test. The Kruskal-Wallis test determined differences between the sorption and solubility groups, and the Mann-Whitney test was applied when needed. The Wilcoxon signed-rank test was used to compare roughness. The statistical significance for all tests was considered $\alpha=0.05$.

RESULTS

The results for the *in vitro* sorption and solubility ($\mu\text{g}/\text{mm}^3$) are shown in Table 3. The Kruskal-Wallis test rejected the null hypothesis for all three storage mediums, indicating that at least one of the groups differed from the rest ($p<0.001$). All tested self-adhering resin composites showed higher and significant sorption for all three immersion media.

The self-adhering resin composites Vertise Flow (VTF) and Fusio Liquid Dentin (FUS) showed the highest sorption for all three immersion mediums. For FUS, statistically significant differences were only observed between lactic acid and artificial saliva. However, results showed that VTF's sorption had no statistical difference between tested media ($p=0.208$). Lower sorption was observed within the bulk-fill composites X-Tra Fil (XTF) and Tetric EvoCeram Bulk Fill (TBF). Their results were comparable to the control group Filtek Z250 (Z250).

The resin composites showed the same behavior for sorption in artificial saliva and lactic acid ($\text{XTF}<\text{TBF}<\text{Z250}<\text{FUS}<\text{VTF}$). Lower sorption was observed for TBF in distilled water ($20.83 \mu\text{g}/\text{mm}^3$). There was no statistical difference between the bulk-fill resin composites and the control group (Z250). However, self-adhering resin composites showed statistical differences between the bulk-fill and control groups ($p=0.009$).

The control group (Z250) showed significantly higher sorption in lactic acid ($p=0.006$) than bulk-fill composites. The sorption of XTF and TBF bulk-fill resin composites did not differ between tested media.

As for solubility, TBF showed the lowest values in all three media. Some high discrepancy values were observed for the bulk-fill resin XTF in artificial saliva ($22.13 \mu\text{g}/\text{mm}^3$) and the self-adhesive FUS in lactic acid ($65.87 \mu\text{g}/\text{mm}^3$). In general, solubility values obtained from the self-adhesive VTF were also higher than the control group.

Table 3: Sorption (S_P) and Solubility (S_L) ($\mu\text{g}/\text{mm}^3$) Means (SD) After 180-Day Storage Period								
Groups	Distilled Water		Artificial Saliva		Lactic Acid		p^*	
	S_P	S_L	S_P	S_L	S_P	S_L	S_P	S_L
Z250	21.46 (2.43) ^{Aa}	4.79 (4.51) ^{Aa}	23.49 (1.18) ^{Ba}	−1.70 (3.28) ^{Ab}	27.46 (1.55) ^{Bb}	5.10 (5.00) ^{ADab}	0.006	0.055
XTF	23.17 (13.54) ^{Aa}	8.65 (8.70) ^{ABa}	14.52 (1.60) ^{Aa}	22.13 (40.50) ^{BDCa}	14.56 (5.04) ^{Aa}	11.99 (3.48) ^{Aa}	0.566	0.221
FUS	50.81 (5.69) ^{Bab}	6.89 (6.24) ^{ACa}	50.46 (3.80) ^{Ca}	7.19 (2.88) ^{Ca}	59.16 (2.30) ^{Cb}	65.87 (6.85) ^{Bb}	0.037	0.009
VTF	62.55 (4.01) ^{Ba}	22.18 (1.94) ^{Ba}	67.26 (3.21) ^{Da}	15.16 (1.24) ^{Db}	64.98 (3.82) ^{Ca}	26.86 (2.78) ^{Cc}	0.208	0.003
TBF	20.83 (2.68) ^{Aa}	−1.56 (3.15) ^{Cac}	20.90 (2.45) ^{Ba}	−6.61 (4.31) ^{Ab}	22.25 (3.02) ^{Ba}	1.69 (1.55) ^{Dc}	0.827	0.009
p value*	0.001	0.005	<0.001	0.001	<0.001	<0.001		
Different superscript letters indicate statistically significant differences between groups through the Mann-Whitney test. Uppercase letters indicate differences between resin composites within each solution (columns); lowercase letters indicate significant differences between solutions (lines).								
* Kruskal-Wallis test.								

In distilled water, VTF and XTF showed significantly higher solubility values of 22.18 and 8.65 $\mu\text{g}/\text{mm}^3$, respectively, than the other materials ($p=0.005$). For artificial saliva, XTF showed the highest sorption value (22.13 $\mu\text{g}/\text{mm}^3$), which was statistically similar to the VTF and FUS values (15.16 and 7.19 $\mu\text{g}/\text{mm}^3$, respectively), but there was a difference between the control group and TBF ($p=0.001$). In general, solubility values obtained from the self-adhesive VTF were also higher than the control groups.

Negative solubility values were seen when TBF (−6.61 $\mu\text{g}/\text{mm}^3$) and Z250 (−1.70 $\mu\text{g}/\text{mm}^3$) were immersed in artificial saliva and when TBF (−1.56 $\mu\text{g}/\text{mm}^3$) was immersed in distilled water. These values can be attributed to the incomplete dehydration of these materials. There was no statistical difference between the tested mediums for XTF ($p=0.221$). However, for VTF, there was a difference for solubility in each tested medium ($p=0.009$).

The results for the *in situ* phase are shown in Table 4. Initially, 20 volunteers were selected. However, only 16 volunteers completed the study. One volunteer presented an allergic reaction in the palatal mucosa, two lost the intraoral palatal device, and one did not complete the established protocol.

Table 4: Mean Surface Roughness (μm) and SD Before and After Biodegradation			
Groups	Initial	Final	p^*
Z250	0.25 (0.08) ^{BC}	0.31 (0.09) ^B	0.001
XF	0.44 (0.15) ^A	0.47 (0.15) ^A	0.278
FLD	0.24 (0.04) ^{BC}	0.28 (0.08) ^B	0.049
VF	0.21 (0.05) ^C	0.28 (0.09) ^B	0.005
TECBF	0.26 (0.09) ^B	0.33 (0.09) ^B	0.049
Different superscript letters indicate differences between groups within each observation moment, according to the Mann-Whitney test.			
* Wilcoxon signed-rank test was used to compare roughness in different moments of observations.			

All the materials showed significantly increased roughness after biodegradation, except for the XTF group ($p=0.278$). The results obtained for the FUS and TBF groups were on the threshold of significance ($p=0.049$). The final roughness of the resin composites tested followed the order: FUS = VTF = Z250 = TBF < XTF.

DISCUSSION

All three null hypotheses were rejected. Differences were observed between the sorption and solubility of the evaluated restorative materials, with different results within storage media. Also, the biofilm interaction resulted in a roughness increase for some of the restorative materials.

Even after polymerization, resin composites are not stable. Different substances may affect their integrity by diffusion into the polymer matrix. The polymer absorbs substances to varying degrees, which are influenced by the polarity of molecular structure, the degree of cross-linking in the continuous matrix, and the presence of hydroxyl groups. The sorption may result in an expansion of the gap between the polymer chains, allowing free unreacted components trapped in the polymer matrix to diffuse out into the storage media. The storage medium and the immersion period strongly influence the sorption and the solubility.^{14,24} Their interaction in the oral cavity with the surface of the restorative materials also favors biodegradation because they are capable of penetrating the polymeric matrix and enhancing the release of the unreacted monomers.²⁵

The solutions used as storage media in this study were chosen because they represent common liquids present in the oral environment. Organic solutions exhibit greater uptake and sorption/solubility values than water-based media.^{14,26,27} In this study, differences in the sorption and the solubility of the evaluated materials was observed within each

storage medium, with higher values when immersed in lactic acid. However, differences between storage media were only found in the sorption of Z250 and FUS. For the solubility, differences were observed in the groups FUS, VTF, and TBF. The lactic acid molecule presents functional groups in its composition such as -OH and -COOH, which may increase interaction with the polar sites of dimethacrylate monomers.¹⁷

Materials that highly absorbed water did not necessarily demonstrate high solubility and vice versa.^{14,28} This phenomenon is attributed to leaching of residual monomers, additives, and filler content, which strongly depends on the degree of conversion, monomer hydrophilicity, mobility and molecular size as smaller molecules will decompose faster.^{24,29-31}

The hydrolytic degradation mechanism is enhanced in the presence of filler particles containing metallic ions (ie, barium and zinc). Some ions are electropositive and tend to react with water. This reaction changes the balance inside the silica network, and the spaces occupied by zinc and barium are reestablished with the penetration of hydrogen ions of water. The siloxane bonds (Si-O-Si) of the silica network start to break with the increase of the concentration of hydroxyl ions, and there is the formation of an autocatalytic cycle of surface degradation that could explain the continuity of superficial softening with aging time.³²

The release of unreacted monomers highly depends on the degree of conversion. It was reported that the degree of conversion of different monomer systems increases in the following order: Bis-GMA < Bis-EMA < UDMA < TEGDMA.³³ The higher the degree of conversion, the lower the number of unreacted monomers, and therefore, the lower the solubility.¹⁴ TEGDMA is a hydrophilic low-molecular-weight monomer with high mobility. It decomposes more quickly than larger molecules, such as Bis-GMA.³⁴ The TEGDMA-resin network has high flexibility and is also more heterogeneous, with larger spaces between the polymers that can therefore accommodate a higher quantity of water inside the micropores.²⁹ Apart from TBF and VTF, all other studied resin composites contain TEGDMA in their composition. UDMA is also present in most of the studied resin composites (except for VTF). UDMA seems to have lower sorption compared with Bis-GMA due to its higher reactivity, degree of conversion,³⁵ and higher resistance to chemical degradation.³⁶ The higher water sorption of Bis-GMA is due to the hydrophilic characteristics of its monomer units. Also, hydroxyl groups form stronger hydrogen

bonds with the water molecules than the urethane groups of UDMA-resin or the ester groups of Bis-EMA resin.³⁷

Monomers with adhesive properties have polymerizable groups with an additional functional group that is linked by a custom-made spacer group. This molecular design influences the hygroscopic expansion of the resulting polymer.^{38,39} The expansion observed in the VTF specimens is due to the presence of a hydrophilic acid phosphate group and a shorter spacer group in the adhesive monomer, GPDM.³⁹ Also, the presence of HEMA makes this material more susceptible to matrix disintegration due to the molecules high hydrophilicity.⁴⁰ As for FUS, besides the adhesive monomer 4-MET, the material contains UDMA, TEGDMA, and HEMA. A previous *in vitro* study⁴¹ evaluated the hygroscopic absorption characteristics of different resin-based materials. Due to the incorporation of hydrophilic monomers, the self-adhering resins were the least dimensionally stable.

Previous studies have confirmed that sorption is highly influenced by the polymeric matrix properties.^{8,14,42-44} Because sorption is a phenomenon associated with the polymeric phase, a negative correlation between sorption and filler loading content⁴⁵ explains that, when weight percentage of fillers increase, the polymeric matrix and the sorption decreases.⁴⁶ The higher sorption values for FUS and VTF could be justified based on their flow characteristics, which require more diluent monomers and filler content. Apart from Z250, the inorganic phase of the evaluated resin composites is based on barium glass and particles such as strontium, which contains leachable ions.³⁹

Resin composites containing barium glasses (XTF, TBF, VTF, FUS) and zirconia silicate fillers (Z250) seem to be more prone to aqueous attack than those containing quartz in their composition. The substantial softening observed in Z250 may be attributed to the smaller surface area and the spherical shape of zirconia/silica fillers that may decrease the filler-matrix bonds.³²

Some materials produced negative solubility values, which can be attributed to incomplete dehydration of the materials. These negative values do not indicate that no solubility occurred but may hint to their low solubility. Possible hydrolytic chemical reactions of glass fillers and metal oxides with water results in the formation of metal hydroxides within the composite.^{14,27} Negative solubility results have also been reported in other studies.^{27,41,47}

The *in vitro* methods can also differ over the need to replace the storage medium.^{17,46,48,49} The replacement would favor the maintenance of a constant pH throughout the experiment.^{14,27} It would be interesting to assess further the variation of the pH on sorption and solubility similar to what occurs in the oral cavity.

In vitro tests are not able to reproduce the complex process of biodegradation. The pH conditions in an *in vitro* environment may be different than observed in the oral conditions.^{1,2} Therefore, *in situ* studies are alternatives to evaluate many situations within the oral cavity in a short period.⁵⁰ The aqueous environment, the salivary properties, the biofilm accumulation, and temperature fluctuations are conditions that can predict the restorative material's behavior⁵¹; lactic acid is the most crucial product metabolized by cariogenic bacteria such as *S. mutans* in the presence of sucrose.⁵² This study model allows the observation of the consequences of biointeraction on the materials' surfaces under constant biodegradation.

Little is known regarding the surface degradation of restorative materials after biofilm interaction *in situ*³ and the exact start time of the degradation process is still uncertain.⁵³ Results are observed at different experimental times: seven days,^{3,54,55} 14 days,⁵⁰ and also periods over 14 days.⁵⁶ A seven-day *in situ* evaluation showed an influence of the biofilm formation on the materials with chemical and physical modifications due to the biodegradation process.^{3,54}

Clinically, the biodegradation process of restorative materials involves the dissolution and disintegration in saliva and other types of physical/chemical degradation. The potential for mechanical and chemical dissolution may cause increased biofilm accumulation.⁵⁰ Most of the tested materials have a combination of BIS-GMA and the diluent monomer TEGDMA in their composition. These monomers are linked by the presence of unprotected esters linkage, which is strongly susceptible to hydrolysis by saliva enzymes generating bis-hydroxy-propoxy-phenyl-propane (BisHPPP), triethylene glycol (TEG), and methacrylic acid (MA). These degradation byproducts have a negative influence on microorganism cell function and metabolism.^{57,58}

The surface roughness increased in all resin composites except for the XTF resin after seven days. The monomer composition can influence bacterial growth, reducing surface degradation by the presence of acid. The presence of TEGDMA

increases the degree of conversion⁵⁹ and reduces the bacterial growth.⁶⁰ Moreover, UDMA presents low biodegradation probably due to the improvement of the chemical resistance.³⁶

The complexity of the degradation process and the difficulty in simulating the intraoral environment *in vitro* shows the need for further analysis that could help understand this complex phenomenon. A resin-based material's surface free energy is a central topic regarding biofilm adhesiveness.¹⁴ Restorative materials with higher surface energy show a decreased resistance to adhesive biofilm formation.⁶¹ *In situ* studies based on caries adjacent to restorative materials still yield inconsistent results,⁵⁶ as many different factors are involved such as distinct diet and oral hygiene regimens, caries risk, salivary flow, and the buffer capacity of saliva.³ It would be interesting for future studies to evaluate the ability of bacterial adhesion and the resulting degradation in the bonding interface of indirect restorations. The interfacial gap formed by acids and bacterial enzymes are sites with a higher level of biodegradation that could lead to interfacial seal compromise, increasing the risk of secondary caries and therefore affecting the clinical longevity.⁵⁷

CONCLUSIONS

All of the resin composites tested demonstrated hydrolytic degradation during 180 days in an *in vitro* environment. However, not all of them showed biodegradation with increased roughness in the *in situ* environment.

The time and storage medium influenced the final characteristics of the resin composites tested. The sorption and solubility seemed to depend on the composition of each material. The self-adhering resin composites showed higher hydrolytic degradation, and the bulk-fill resin composites had similar behavior to the conventional microhybrid resin composite used as a control. The XTF resin presented higher solubility in the *in vitro* environment. However, it showed chemical resistance in the *in situ* environment.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee

guidelines and policies of the Local Ethics Committee from University of Pernambuco. The approval code for this study is 38778814.1.0000.5207.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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An *In Vitro* Investigation of Anticaries Efficacy of Fluoride Varnishes

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Clinical Relevance

This study provides clinicians with insight into the *in vitro* differences in the performance of commercially available fluoride varnishes. More clinical research is needed to equip dentists with evidence that will help them make better treatment choices.

SUMMARY

Most currently marketed fluoride varnishes (FVs) have not been evaluated for their effectiveness in preventing dental caries. The objective of this study was to investigate the anticaries efficacy, measured as fluoride release into artificial saliva (AS); change in surface microhardness of early enamel caries lesions; and enamel fluoride uptake (EFU) of 14 commercially available FVs and two control groups. Bovine enamel specimens (5×5 mm)

were prepared and assigned to 18 groups (n=12). Early caries lesions were created in the specimens and characterized using Vickers microhardness (VHN_{lesion}). FV was applied to each group of specimens. Immediately afterward, specimens were incubated in 4 mL of AS for 18 hours, which were collected and renewed every hour for the first six hours. AS samples were analyzed for fluoride using an ion-specific electrode. Specimens were then brushed for 20 seconds with toothpaste slurry and subjected to pH cycling consisting of a four-hour/day acid challenge and one-minute treatments with 1100 ppm F dentifrice for five days. Microhardness was measured following pH cycling (VHN_{post}). EFU was determined using microbiopsy. Acid resistance (eight-hour demin challenge) was performed after pH cycling, and microhardness was measured (VHN_{art}) and compared with baseline values to test the FV impact after pH cycling. One-way analysis of variance was used for data analysis ($\alpha=0.05$). FVs differed in their release characteristics (mean $\pm$ SD ranged from 14.97 ± 2.38 $\mu\text{g/mL}$ to 0.50 ± 0.15 $\mu\text{g/mL}$), rehardening capability (mean $\pm$ SD ranged from 24.3 ± 15.1 to 11.7 ± 12.7), and ability to deliver fluoride to demineralized lesions (mean $\pm$ SD ranged from

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3303 $\pm$ 789 $\mu\text{g}/\text{cm}^3$ to 707 $\pm$ 238 $\mu\text{g}/\text{cm}^3$). Statistically significant but weak linear associations were found between $\Delta\text{VHN}_{(\text{post} - \text{lesion})}$, EFU, and fluoride release (correlations 0.21-0.36). The results of this study demonstrated that differences in FV composition can affect their efficacy in *in vitro* conditions.

INTRODUCTION

The effects of topically applied fluoride treatments have been well known since the 1930s and have resulted in beneficial outcomes in caries reduction.¹⁻⁵ Several studies have confirmed the effectiveness of fluoride varnishes (FVs) in reducing caries incidence and prevalence with the added benefits of ease of use and, most importantly, virtually no side effects.⁶⁻⁸

In vitro models are very commonly used in cariology research. Most established models are designed to mimic clinical conditions as closely as possible. *In vitro* models have controlled scientific settings that limit variability and increase sensitivity compared with clinical models. However, they come with their own limitations in their inability to fully simulate the complex intraoral environment, especially circumstances leading to formation of dental caries.⁹⁻¹¹

Most of the available *in vitro* studies that investigate the efficacy of FVs vary in their experimental design and testing conditions.^{12,13} This creates heterogeneity, making it difficult to find benchmark tests that accurately assess efficacy. Therefore, we have selected to investigate different research variables that are of importance when studying fluoride efficacy in an attempt to improve our understanding of FVs and find the best test conditions. We also tried to study how those variables relate to each other in an effort to establish relevant variables for *in vitro* FV research.

The aim of our study was to investigate the potential anticaries effect of 14 commercially available FVs on artificially created early caries lesions through the following outcome variables: 1) 24-hour fluoride release into artificial saliva (AS), b) surface microhardness, and c) enamel fluoride uptake (EFU), after pH cycling for five days with a second demineralization challenge.

METHODS AND MATERIALS

Specimen Preparation

Enamel specimens, 5 $\times$ 5 mm, were obtained from bovine teeth using a low-speed saw (IsoMet, Buehler, Lake Bluff, IL, USA). The teeth were stored in

deionized water saturated with thymol during the sample preparation process. The specimens were ground and polished to create flat surfaces to facilitate surface microhardness testing using the Struers RotoPol 31/RotoForce 4 polishing unit (Struers Inc, Cleveland, PA, USA). The bottom side of the specimens was ground flat to a uniform-thickness using 500-grit silicon carbide grinding paper. The topside of the specimens was ground in a series of 1200-, 2400-, and 4000-grit paper until most of the tooth surface was flattened. The specimens were then polished using 1- μm diamond paste to eliminate any scratches that may have resulted from the grinding procedure. The specimens were sonicated in deionized water between each grinding/polishing step. As a final cleaning step, the polished specimens were sonicated in 2% micro-liquid. The specimens were assessed with a magnification of 10 $\times$.

To be acceptable for the study, a specimen was required to

- 1) have a minimum 5 $\times$ 5 mm polished facet across the surface;
- 2) not have any obvious cracks or other flaws in the enamel surface;
- 3) have an evenly polished, high-gloss enamel surface; and
- 4) have no contamination on the top surface from sticky wax or any other material.

Specimens were then secured, with the polished enamel side facing upward, on a one-inch square acrylic block using sticky wax to facilitate surface microhardness measurements. Then, all specimen surface areas apart from the polished top surface were covered with a colored nail varnish (Sally Hansen Advanced, Hard As Nails Nail Polish, Red, New York, NY, USA). Prepared specimens were stored at approximately 100% relative humidity and 4°C until further use. A total of 216 specimens were prepared for the present study (18 groups of 12 specimens each).

Sound Enamel Microhardness

Initial surface microhardness of the sound enamel specimens was determined using a Vickers microhardness indenter at a load of 200 g for 11 seconds (Instron T2100B Vickers Surface Microhardness Tester, Norwood, MA, USA). The average sound enamel microhardness ($\text{VHN}_{\text{sound}}$) was determined from five indentations on the surface of each specimen (Figure 1). Only specimens with $300 \leq \text{VHN}_{\text{sound}} \leq 400$ were accepted into the study.

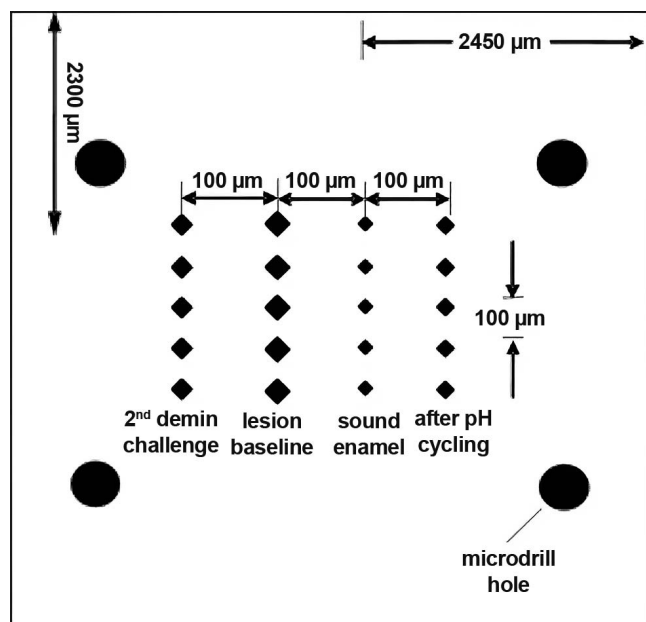


Figure 1. Enamel specimen (5×5 mm) with microhardness indentations and microdrill holes.

Artificial Lesion Creation

Artificial lesions were formed in the enamel specimens by a 48-hour immersion at 37°C into a solution of 0.1 M lactic acid and 0.2% Carbopol C907, which was 50% saturated with hydroxyapatite and adjusted to pH 5.0 using KOH. After demineralization, the specimens were rinsed with deionized water for approximately one minute. Specimens were blotted dry with a tissue and stored at approximately 100% relative humidity and 4°C until further use.

Lesion Baseline Microhardness

Microhardness of the demineralized enamel specimens was determined as described above. The average specimen lesion baseline microhardness (VHN_{lesion}) was calculated. Only specimens with $25 \leq VHN_{\text{lesion}} \leq 60$ were accepted into the study. Specimens were assigned to treatment groups ($n=12$) based on a randomization procedure that resulted in treatment groups with mean VHN_{lesion} values that were not significantly different statistically.

Specimen Mounting

Once assigned to their treatment groups, specimens were removed from their acrylic blocks and mounted onto a 12-well microtiter plate lid. Acrylic blocks (12×12×9 mm) were mounted onto the inside of the

lid using acrylic glue. Then, specimens were mounted with the enamel side facing upward onto the acrylic block using sticky wax.

FV Application

All products tested in this study contained 5% NaF (22,600 ppm fluoride ion) and were assigned to groups and labeled as per Table 1. In addition to the test FVs shown above, two additional experimental groups were included: one placebo group (O−; placebo varnish, no toothpaste treatment during pH cycling phase) and one positive control group (O+; no FV treatment, toothpaste treatment during pH cycling phase). The placebo varnish had the following composition (all w/w): 2% shellac, 10% ethyl cellulose, 40% ethyl acetate, 2% polyvinylpyrrolidone, 2% xylitol, 5% NaCl, and 39% ethanol and was manufactured in-house especially for this study.

The protective foil from the individual FV dose was removed, and the FV was mixed using the manufacturer's applicator for at least 10 seconds to homogenize the FV, as sedimentation of NaF and phase separation may have occurred during storage. Duraphat (group C) was supplied in a tube. For this FV, approximately 0.5 mL was squeezed into a small weighing cup and processed as described above. The placebo varnish was handled in a similar manner.

Subsequently, FV was applied to the surface of each specimen using a single brush stroke and using the manufacturer's applicator. Any unused FV was discarded.

Saliva Incubation

Immediately after FV application, the lid containing 12 specimens was placed onto the microtiter plate containing 4.0 mL of AS per well. AS had the same composition as in a previous experiment.¹⁴ Two batches of this solution were prepared: one for the FV incubation phase in all specimens and one for the pH cycling phase for all specimens.

The microtiter plate was then placed in an incubator set at 37°C. Every hour for six hours, the lid was placed onto a new microtiter plate containing 4.0 mL fresh AS per well.

This procedure was repeated until a total AS exposure time of six hours had been reached. After six hours, the lid was placed onto a new microtiter plate containing 4.0 mL AS per well, which was placed in the incubator for 18 hours. All saliva samples were frozen immediately after each cycle and retained for analysis of ionic fluoride.

Table 1: Study Test Products

Group	Product	Manufacturer	Noteworthy Ingredient
A	CavityShield 5% Varnish	3M ESPE	—
B	Vanish 5% NaF Varnish w/TCP	3M ESPE	Functionalized tricalcium phosphate (fTCP), Xylitol
C	Colgate Duraphat Varnish	Colgate Oral Pharmaceuticals	—
D	Colgate Prevident Varnish	Colgate Oral Pharmaceuticals	Xylitol
E	Sparkle V Varnish	Crosstex International	Xylitol
F	Nupro 5% Fluoride Varnish	Dentsply Professional Division	—
G	Kolorz Clearshield Varnish	DMG America	Xylitol
H	MI Varnish	GC America	Casein phosphopeptide-ACP (CPP-ACP, Recaldent)
I	Duraflor Halo 5% Sodium Fluoride Varnish	Medicom	—
J	Enamel Pro Varnish Clear	Premier Dental	Amorphous calcium phosphate (ACP), Xylitol
K	Vella Fluoride Varnish	Preventive Technologies	Xylitol
L	Butler White Fluoride Varnish	Sunstar Americas, Inc	Xylitol
M	Flor-Opal Varnish White Fluoride Varnish	Ultradent	Xylitol
N	Waterpik UltraThin Varnish	Waterpik Technologies Inc	Xylitol
O	Placebo Varnish	(manufactured in-house)	—

Then, a soft toothbrush (Oral B P40, Procter & Gamble, Mason, OH, USA) was used to brush each specimen by hand. A slurry of Crest Cavity Protection (0.243% sodium fluoride; Procter & Gamble) at a ratio of 1:2 w/w (dentifrice: AS) was prepared in a beaker with a magnetic stirrer. The toothbrush was dipped into the slurry briefly (approximately two seconds). Each specimen was then brushed under a stream of deionized water for 20 seconds, with the specimen being rinsed another 10 seconds after brushing. This procedure was repeated until all specimens on the lid were brushed. A new toothbrush and slurry was used for each lid. After this procedure, the pH cycling phase commenced on the same day.

pH Cycling Phase: Remineralization

The present study was separated into three phases, and each phase contained a placebo group (ie, O-a, O-b, O-c) to allow for comparisons between phases. The daily cyclic treatment regimen for each lid containing the specimens consisted of one, one-minute treatment period with a slurry of aforementioned toothpaste (prepared as described above), followed by a two-hour exposure to AS, four hours per day acid challenge in the lesion-forming solution, and exposure to AS for the remainder of the day and overnight. One slurry per day was prepared and pipetted into each well of the used microtiter plates.

The pH cycling was performed by placing the lid containing the specimens onto different microtiter plates containing 4.0 mL each per well of toothpaste

slurry, AS, or lesion-forming solution. After each treatment, the specimens were rinsed under running deionized water briefly (approximately two seconds per specimen). The regimen was repeated for five days. The study was conducted at room temperature.

After completion of the pH cycling phase, all specimens were carefully removed from the lids and remounted onto an acrylic block to facilitate microhardness and EFU measurements.

Posttreatment Microhardness

The average specimen microhardness (VHN_{post}) was determined again in the same manner as when obtaining lesion and baseline microhardness.

Enamel Fluoride Uptake

The fluoride content of the enamel specimens was determined using the microdrill technique. The enamel specimens were mounted perpendicular to the long axis of a micro end mill attached to a specially designed microdrill and drilled to a depth of 100 μ m through the entire lesion. The drilling and sample collection were performed in a static-controlled atmosphere to prevent loss of enamel powder due to charging effects. The enamel powder sample was transferred to a diffusion dish and then analyzed for fluoride. The diameter of the drill hole was determined using a calibrated microscope interfaced with an image analysis system. Indentations for microhardness testing and microdrill holes were placed on enamel specimens according to Figure 1.

Acid Resistance Test

To test whether the various FVs impart acid resistance to the enamel specimens after pH cycling, a second *in vitro* demineralization treatment was performed and followed the same protocol as described above but using a demineralization time of only eight hours. The average specimen microhardness (VHN_{art}) was determined again as described above.

AS Fluoride Analysis

The collected, frozen AS samples were thawed. An aliquot was removed and analyzed for fluoride by comparison to a similarly prepared standard curve using an ion-selective electrode (Model 9609BNWP, Orion Research, Boston, MA, USA) and meter. Individual as well as cumulative F release data were calculated to determine $[F]_{max}$ (highest F concentration found in any collected AS sample over the 24-hour period) as well as F_{total} (the total amount of F released from FV).

Statistical Analysis

A one-way analysis of variance statistical method was used for data analysis. An overall significance of ($\alpha=0.05$) was used. Pairwise comparisons between the groups were conducted using Tukey's method. Pearson correlation coefficients were calculated to investigate associations between the study variables.

RESULTS

Specimens treated with Nupro had the highest $\Delta VHN_{(post - lesion)}$, ranging from 24.3 for Nupro to 11.7 for Vella for the specimens receiving active treatment (Figure 2). No significant differences were found among FV; however, Nupro was the only FV significantly different from all placebo varnishes.

Specimens treated with Sparkle had the highest rehardening values following the second acid challenge, $\Delta VHN_{(art - lesion)}$, but were significantly different only from those treated with Vella. All other FVs did not statistically affect lesion rehardening differently, but all FVs were significantly different from the placebo varnishes (Figure 3).

Fluoride release trends were somewhat similar for all tested FVs (Figure 4). The highest release for most FVs was within the first hour of testing, and then varnishes exhibited a steady decrease in fluoride release over time. MI released significantly more fluoride in the first hour than all the other test products (9.71 $\mu\text{g/mL}$). Enamel Pro came

second (5.44 $\mu\text{g/mL}$), followed by Flor Opal (4.37 $\mu\text{g/mL}$); however, the latter two were not statistically different from each other. Flor Opal released in the first hour of the experiment approximately half that of MI. Butler White released significantly less fluoride (0.12 $\mu\text{g/mL}$) in the first hour than all other test products. Vella and Kolorz demonstrated atypical release behavior where peak release occurred at the five-hour collection point (2.55 $\mu\text{g/mL}$).

For fluoride release total (F_{total}), MI released significantly more fluoride over the 24-hour incubation period of the experiment ($F_{total}=14.97$ $\mu\text{g/mL}$) than all other FVs (Table 2). In addition, Enamel Pro and Flor Opal released more fluoride than the remaining FVs, except Nupro. Butler White released significantly less fluoride ($F_{total}=0.50$ $\mu\text{g/mL}$) than all other FVs.

The highest concentration of fluoride at any given time point was for MI ($[F]_{max}=9.71$), which was significantly higher than all FVs except for Enamel Pro ($[F]_{max}=5.44$), with Enamel Pro and Flor Opal having higher peak concentrations than all remaining FVs. Butler White exhibited the lowest peak concentration ($[F]_{max}=0.17$) and was significantly lower than all other FVs under study (Table 2).

Differences between FVs were less prominent for EFU than for F_{total} . PreviDent, Duraphat, and Vanish had significantly higher EFU than Cavity Shield, MI, Flor Opal, and Butler White but were not significantly different from the other FVs (Figure 5).

There were statistically significant but weak linear associations between $\Delta VHN_{(post - lesion)}$ and EFU ($r=0.36$; $p<0.0001$), $\Delta VHN_{(post - lesion)}$ and F_{total} ($r=0.22$; $p=0.0011$), and F_{total} and EFU ($r=0.21$; $p=0.0022$).

DISCUSSION

In this *in vitro* study, we aimed to investigate the efficacy of 14 commercially available FVs using the same outcome variables from one of our previous *in vitro* investigations.¹⁴ While the two studies investigated the effect of FVs on caries lesions, the study models were fundamentally different. The wide variation in performance of FV in our first experiment prompted the design of this *in vitro* study. We aspired to have a better understating of how different formulations affect the efficacy of FVs. Therefore, in this experiment, we aimed to investigate a larger variety of FVs in an attempt to have a range of products that represent different formulations. Also, in this study, FVs were left on the

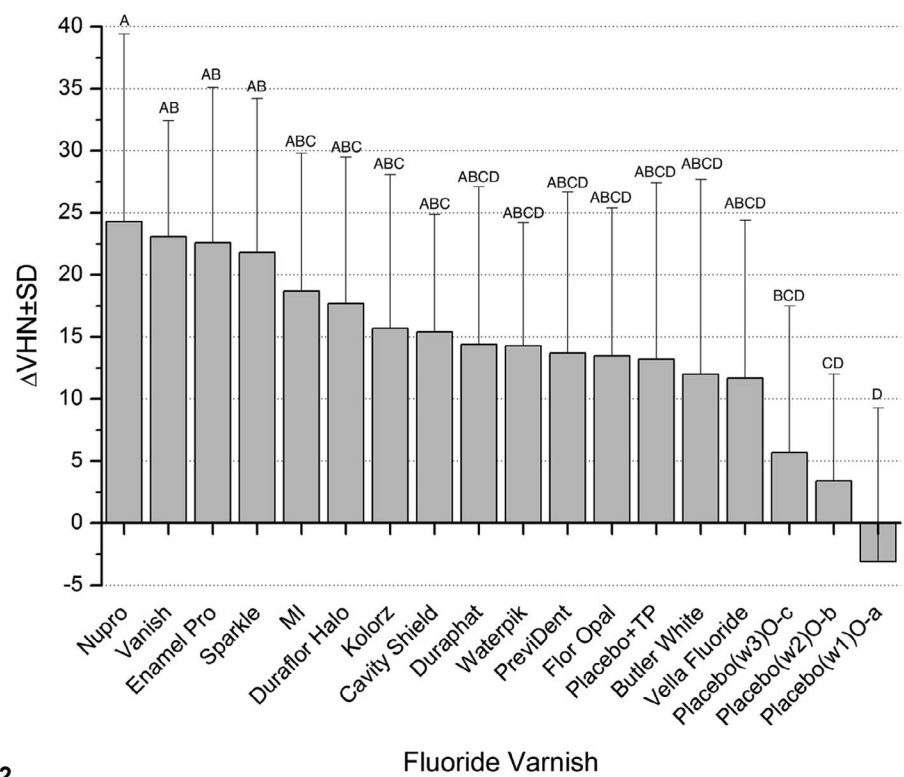
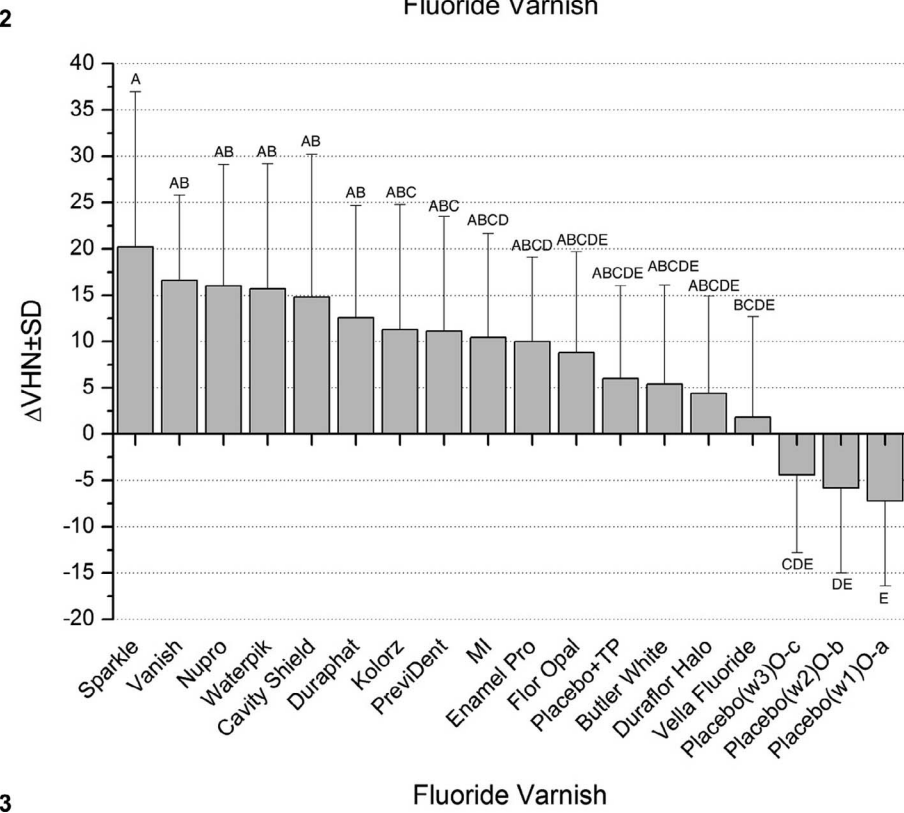
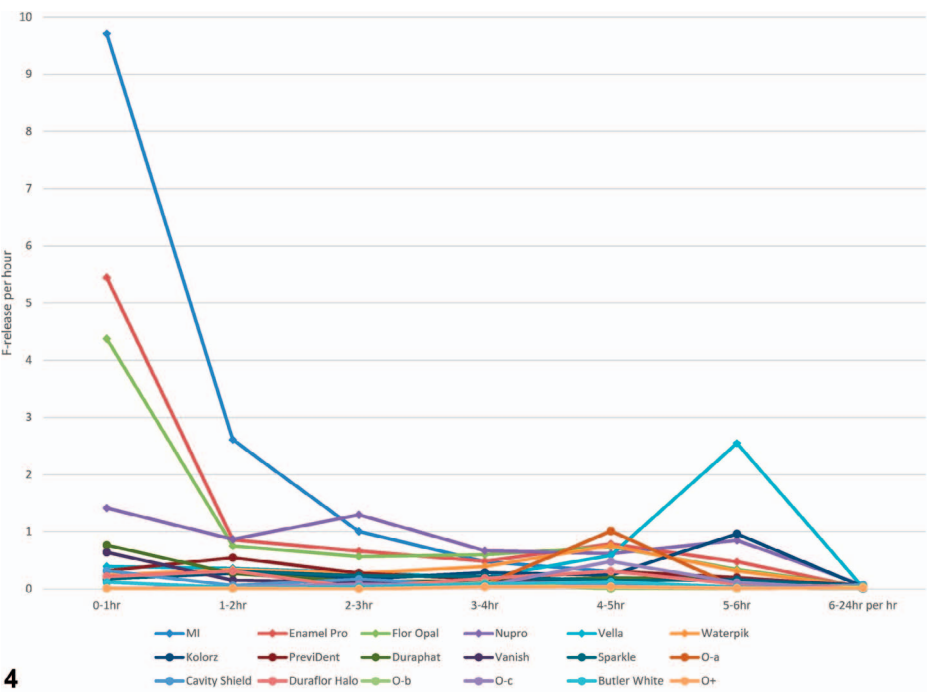


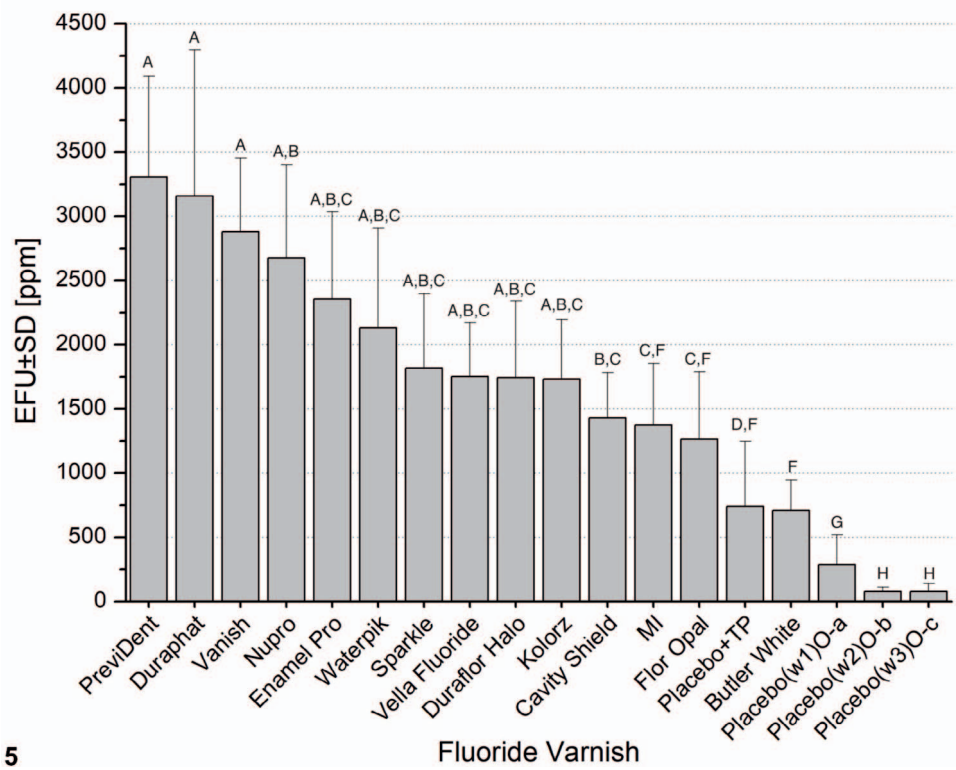
Figure 2. Mean change in surface microhardness ($\Delta VHN_{(post - lesion)}$) as a function of fluoride varnish treatment. Significant differences between varnishes are highlighted by different letters. Error bars denote standard deviations.

Figure 3. Mean change in surface microhardness ($\Delta VHN_{(art - lesion)}$) as a function of fluoride varnish treatment. Significant differences between varnishes are highlighted by different letters. Error bars denote standard deviations.





4



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specimens for a prolonged period of 24 hours versus the six-hour period of the first study to better simulate the clinical situation.

This *in vitro* experiment's results for $\Delta VHN_{(post - lesion)}$ and for $\Delta VHN_{(art - lesion)}$ were unanticipated. As there are numerical differences in lesion rehar-

dening values from different FVs, most of the FVs did not statistically differ from each other. Unfortunately, it is hard to thoroughly interpret the results because of the lack of detailed information on different FV formulations. However, there seems to be a superior effect on lesion rehardening when

Figure 4. Mean hourly fluoride release from fluoride varnishes into saliva.

Figure 5. Enamel fluoride uptake (EFU) as a function of fluoride varnish treatment. Different letters highlight significant differences between varnishes. Error bars denote standard deviations.

Table 2: Cumulative Fluoride Release and Peak Fluoride Concentration (n=12)^a

Fluoride Varnish	Cumulative Fluoride Release [µg/mL], Mean (SD)	Peak Fluoride Concentration [µg/mL], Mean (SD)
MI	14.97 (2.38) A	9.71 (1.40) A
Enamel Pro	9.20 (1.71) B	5.44 (1.05) A,B
Flor Opal	8.20 (1.91) B	4.37 (0.85) B
Nupro	6.96 (1.26) B,C	1.69 (0.43) C,D
Vella	4.91 (2.02) C,D	2.57 (2.17) C
Waterpik	3.07 (0.81) D,E	0.93 (0.30) D,E
Kolorz	2.91 (0.46) D,E	1.00 (0.11) C,D,E
PreviDent	2.82 (0.59) E,F	0.98 (0.09) C,D,E
Duraphat	2.64 (0.53) E,F	0.97 (0.12) C,D,E
Vanish	2.63 (0.53) E,F	1.28 (0.09) C,D,E
Sparkle	2.19 (0.47) E,F	0.95 (0.15) C,D,E
O-a	2.15 (0.56) E,F	1.20 (0.30) C,D,E
CavityShield	1.97 (0.14) E,F	1.28 (0.11) C,D,E
Duraflor Halo	1.82 (0.54) F	0.70 (0.22) E
O-b	0.85 (0.17) G	0.71 (0.16) E
O-c	0.81 (0.55) G,H,I	0.55 (0.51) F
Butler White	0.50 (0.15) H,I	0.17 (0.09) H
O+	0.49 (0.16) I	0.37 (0.16) G

^a Significant differences between varnishes are highlighted by different letters.

calcium- and phosphate-containing ingredients are added to the formulation. This is in agreement with our previous *in vitro* study.¹⁴ This may be attributed to the abundant amount of calcium and phosphate from these formulations that is readily available to interact with the teeth. It is important to note that the enhanced rehardening effect from calcium- and phosphate-containing ingredients did not withstand the second acid challenge. This is in contrast to a recent systematic review that suggested a possible long-term effect (>three months) of casein phosphopeptide-ACP (CPP-ACP) complexes on early caries lesions; however, the results cannot be extrapolated as the review investigated CPP-ACP alone because of insufficient evidence on the complex's synergistic effect with fluoride.¹⁵ Another noteworthy finding is that lesions treated with Vanish, an FV-containing functionalized tricalcium phosphate (fTCP), had a higher $\Delta VHN_{(post - lesion)}$ than those treated with FVs containing ACP and CPP-ACP. This may be due to the protective effect of functionalization of the TCP molecule that prevents premature interaction between calcium and fluoride and aids in remineralization in a manner similar to that of fluoride.¹⁶

The fluoride release data in our experiment are in agreement with our previously mentioned in-house experiment and the 2014 study by Cochrane and others.^{12,13} Calcium-containing FVs, with the excep-

tion of fTCP-containing varnishes, were able to release significantly higher levels of fluoride into saliva. This suggests a synergistic effect of adding casein complexes to FVs on fluoride release and may be explained by the bioavailable nature of ACP and CPP-ACP compared with the less-soluble fTCP.¹⁶⁻¹⁸

EFU data from this study extend our results from our aforementioned *in vitro* investigation. Once again, a CPP-ACP-containing varnish delivered less fluoride into caries lesions. This may be a result of the negative impact that inorganic phosphate released from CPP-ACP varnishes has on the formation of CaF_2 , thus reducing bioavailable fluoride. Vanish, an FV-containing fTCP, delivered more fluoride into lesions compared with CPP-ACP and ACP-containing FV despite its low fluoride release into saliva. This is in agreement with a study that compared EFU from two varnishes, one containing fTCP and the other containing CPP-ACP.¹⁷ Also, another study compared EFU with and without fTCP. It was found that lesions exposed to fluoride in conjunction with fTCP had significantly higher fluoride uptake than those exposed to fluoride alone, and the effect of fTCP was dose dependent. The mechanism of action of fTCP on enhancing lesion uptake of fluoride is not fully understood but may be attributed to the ability of fTCP to promote fluoride-based nucleation.¹⁶

Contrary to our findings in our preceding *in vitro* study, we were able to demonstrate a significant but weak linear association between $\Delta\text{VHN}_{(\text{post} - \text{lesion})}$ and EFU. To the best of our knowledge, this is the first time an association was established between ΔVHN and EFU in FV research. We are also in agreement with another study in the lack of meaningful correlation between fluoride release and EFU.¹⁹ Based on our findings, we believe that fluoride release cannot be used as a predictive measure for EFU, and therefore, one should be careful in relating fluoride release to FV efficacy.

While our findings are in agreement with some studies and in disagreement with others, it is important to remember that all FV *in vitro* models have their limitations to the extent that the *in vivo* situation cannot be mimicked because of the complexity of *in vivo* caries. With FV in particular, there is the issue of infrequent applications, which makes modeling much harder. In our model, we tried to mimic the *in vivo* application more closely. Most other models measure only the direct effect of an FV treatment while ignoring the effect of topical fluorides applied between FV applications. This makes our study more clinically relevant but also more challenging, as there are competing effects of the topical fluorides.

CONCLUSION

The results of this study demonstrated variation in FV efficacy in *in vitro* conditions, most likely because of differences in composition. Further investigations are needed to develop models that will help us understand FV behavior.

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Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Mechanical Behavior of Ceramic Monolithic Systems With Different Thicknesses

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Clinical Relevance

Highly translucent fully stabilized zirconia can be less fracture resistant than lithium disilicate and partially stabilized zirconia depending on the thickness.

SUMMARY

Objectives: This study assessed the fully stabilized zirconia (FSZ) Prettau Anterior, the partially stabilized zirconia (PSZ) Prettau, and the lithium disilicate IPS e.max CAD (LD) through microstructural and mechanical char-

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acterization and effect of thickness on fracture load of the ceramics.

Methods and Materials: Disk-shaped specimens (12 mm diameter and 1.2 mm thickness) were prepared for biaxial flexural strength (BFS) and Weibull statistics (n=30). For the fracture load static test (FLST) and Weibull statistics (n=30), disk-shaped specimens 12 mm in diameter and thicknesses of 0.5 mm, 1 mm, and 1.5 mm were cemented on an epoxy-resin substrate.

Results: BFS (MPa) results were PSZ: 683.0 ± 70.23 ; FSZ: 438.6 ± 64.1 ; and LD: 248.6 ± 37.3 . One-way analysis of variance (ANOVA) for BFS was significant ($p < 0.001$), and the Tukey post hoc test showed differences among all ceramics. There was difference in characteristic strength, but there was no difference in Weibull modulus. Two-way ANOVA for FLST was significant for ceramic ($p < 0.0001$), thickness ($p < 0.0001$), and interaction ($p < 0.0001$). There was no difference among all ceramics at the 0.5 mm thickness. PSZ had higher values for the 1.0 mm and 1.5 mm thicknesses. LD of 1.5 mm thickness exhibited a higher FLST than FSZ.

Conclusions: PSZ had the highest BFS, but when cemented on a substrate, all ceramics

with 0.5 mm thickness behaved similarly. Despite the lower BFS, LD had a fracture load similar or superior to FSZ when cemented on a substrate.

INTRODUCTION

Veneer chipping is the most common cause of failure in all-ceramic restorations.¹⁻⁹ This failure occurs mainly because of residual tension developed during cooling in firing schedules due to differences in thermal properties between substructure ceramics and porcelain veneers.^{3,8,10,11}

Monolithic materials were developed to avoid the use of veneer porcelain in all-ceramic restorations. Among these materials, lithium disilicate ceramics are the most popular because of their high translucency, strength,¹² and relative simplicity of the technique¹³; they also have a survival rate up to 100% in two-year clinical usage for single crowns.¹⁴ However, the survival rate of fixed partial dentures does not exceed 84% after two-years of clinical use, and the rate is 78.1% after a five-year clinical follow-up.¹⁴ An alternative is monolithic zirconia with higher translucency and color techniques, aiming to associate high strength with better optical properties.^{15,16}

The high opacity of conventional zirconia is due to light-scattering factors (alumina used as a sintering additive, oxygen vacancies, porosity, grain size),¹⁷⁻¹⁹ as well as the intrinsic characteristics of tetragonal zirconia crystals, which are optically anisotropic, where refraction occurs in different crystallographic directions. Conventional yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) grains have sizes in the range of 0.2 to 0.8 μm , and because translucency is dependent on the number of grain boundaries, Y-TZPs with a smaller grain size have more grain boundaries and, thus, tend to have lower translucency because light refracts at each intersecting grain boundary. Larger tetragonal grains have fewer grain boundary areas; however, these are more susceptible to low temperature degradation.²⁰ One approach to reduce the birefringence is to use specific tetragonal grain sizes²¹ only slightly larger than conventional ones, and with reduced alumina concentrations. An opposite approach to obtain translucent Y-TZP is by using nano-scale grain size (<100 nm) because once they are smaller than the wavelength range of visible light (400-700 nm), it is possible to achieve almost transparent ceramics.²¹

Another strategy to increase the translucency of monolithic zirconia is the development of fully

stabilized zirconia (FSZ), acclaimed as the most translucent zirconia. While the partially stabilized zirconia (PSZ) tetragonal phase is stabilized with 2-5 mol% yttria content, in FSZ, yttria is present in concentrations >8 mol%.^{22,23} The higher availability of yttria leads to increased cubic phase concentration.²² Cubic grains have isotropic optical refraction, and they are larger than tetragonal crystals (ie, fewer grain boundary interceptions).

Monolithic systems allow minimally invasive tooth preparation compared with preparations for restorations with porcelain veneering.^{24,25} This reduces the pulp damage risk^{24,26} and allows for tooth preparation with higher axial height.²⁷ Usually, it is recommended that a tooth be reduced by 1.5 mm at the occlusal area for a lithium disilicate restoration, but there is uncertainty around how much smaller the occlusal reduction could be for a stronger ceramic, such as monolithic zirconia, and if a thinner zirconia restoration could be stronger and as translucent as a thicker lithium disilicate restoration.^{24,26,28-31} It has been shown that monolithic Y-TZP crowns with 1 mm thickness possess strength similar to 1.5 mm thick porcelain fused to metal crowns.²⁶ Another study found that monolithic zirconia crowns of 0.6 mm thickness had a fracture load similar to lithium disilicate crowns, which were 1.5 mm thick.³² Some studies using PSZ indicate that a thickness of 0.5 mm at the occlusal face in monolithic zirconia is enough to resist fracture in the molar region.^{24,26,27,31,33} However, FSZ is different from PSZ because translucency is obtained by changes in composition and microstructure that may impair mechanical properties.³⁴

The clinical success of ceramics is dependent on mechanical resistance.³⁵ According to the ISO 6872:2015,³⁶ flexural strength is the selected testing for mechanical evaluation, which is mainly indicated for materials with brittle behavior, typical of ceramics. Nevertheless, mechanical testing for ceramics results in a wide range of tension values because of the effect of internal defects, as pores and inclusions.³⁷ The material is more reliable when the stress values are less dispersed.³⁷ A way to evaluate the reliability is to calculate the Weibull modulus and characteristic resistance.³⁷

In addition, it is important to understand how those ceramic materials behave in a functional simulation. An all-ceramic restoration is bonded to a dental substrate, and the mechanical performance can be modulated by the material's modulus of elasticity, cement properties, adhesion to the substrate, and restoration thickness.³⁸

The esthetic and mechanical behavior of lithium disilicate, and mechanical properties of translucent PSZ are relatively well understood.^{12,39,40} However, considering that FSZ is more translucent than PSZ, but presumably less resistant,³⁴ this study aims to characterize the mechanical properties and microstructure of a lithium disilicate, a translucent PSZ, and a highly translucent FSZ, as well as the fracture load of different ceramic thicknesses bonded to an epoxy-resin substrate. The null hypothesis was that there is no statistical difference between the materials in relation to biaxial flexural strength (BFS) and fracture load of bonded specimens in different thicknesses.

METHODS AND MATERIALS

Three monolithic ceramics were investigated: 1) PSZ Prettau (Zirkonzahn GmbH, Bruneck, Italy), 2) FSZ Prettau Anterior (Zirkonzahn GmbH), and 3) lithium disilicate IPS e.max CAD (Ivoclar Vivadent, Schaan, Liechtenstein).

BFS Testing, Weibull Analysis, and Scanning Electron Microscopy

Zirconia disks (PSZ and FSZ) were milled 20% larger than the final dimensions required for BFS testing according to ISO 6872:2015.³⁶ Specimens were smoothed with silicon carbide grinding paper 1200, 2400, and 4000 (Struers LaboPol 21, Struers, Rodovre, Denmark),⁴¹ ultrasonically cleaned in distilled water for 15 minutes, and dried for 2 hours at 37 °C. Sintering was conducted according to the manufacturer's instructions, and final specimens were 12 mm in diameter and 1.2 mm thick.

Lithium disilicate IPS e.max CAD LT (Low Translucency) A2 samples were prepared in a manner similar to zirconia specimens, but they were cut in the final dimensions (12 mm diameter and 1.2 mm thickness), as there is no change in dimensions after sintering according to the manufacturer's instructions.³⁹

BFS testing (piston on three balls) was carried out according to ISO 6872:2015³⁶ for dental ceramics, performed in a universal testing machine (MTS 810 – MTS System Corporation, Eden Prairie, MN, USA) at a crosshead speed of 1.0 mm/min until failure. The specimens (n=30) were placed concentrically on three hardened steel balls with 2.5 mm diameter, and positioned 120° apart on a support circle with a diameter of 10 mm. The load was applied with a flat punch with a diameter of 1.4 mm at the center of the specimen. The load to failure was registered in N

and then calculated in MPa according to the following equation (ISO 6872:2015)³⁶:

$$S = -0.2387 \frac{P(X - Y)}{d^2}$$

where S represents the maximum tensile stress in MPa, P is the total load to fracture in Newtons, and d is the specimen thickness (mm). The X and Y values were obtained as follows:

$$X = (1 + \nu) \ln \left(\frac{r_2}{r_3} \right)^2 + \left[\left(\frac{1 - \nu}{2} \right) \right] \left(\frac{r_2}{r_3} \right)^2$$

$$Y = (1 + \nu) \left[1 + \ln \left(\frac{r_2}{r_3} \right)^2 \right] + (1 - \nu) \left(\frac{r_1}{r_3} \right)^2$$

where ν is the Poisson ratio (= 0.23), r_1 is the radius of the support circle, r_2 is the radius of the loaded area, and r_3 is the radius of the specimen (all radii expressed in millimeters).

Weibull regression analysis was performed based on the flexural strength data to determine the Weibull modulus and characteristic strength. The Weibull distributions were calculated according to the following equation:

$$P = 1 - \exp \left[\left(-\frac{\sigma}{\sigma_0} \right)^m \right]$$

where P is the probability of fracture, σ is the flexural strength, σ_0 is the characteristic strength at a fracture probability of 63.21%, and m is the Weibull modulus, which is the slope of the line plotted on the “ln(ln [1/(1-P)]) vs ln σ ” Cartesian plane.

$$\ln \left(\frac{1}{1 - P} \right) = m \ln \sigma - m \ln \sigma_0$$

The correction factor was used to adjust the sample size following the DIN 51 110 standard.

Surface analyses were performed by scanning electron microscopy (SEM; JMS-T33A Scanning Microscope, JEOL USA Incorporation, Peabody, MA, USA) on three representative samples for each material and treatment. The specimens of lithium disilicate were etched with 10% hydrofluoric acid for 60 seconds. The zirconia specimens were heated to 1400°C for 30 minutes to reveal the microstructure.⁴² The following parameters were used: low

Table 1: <i>Biaxial Flexural Strength (MPa), Characteristic Strength and Weibull Modulus With a Confidence Interval of 95% for the Materials^a</i>					
Material	BFS	Characteristic Strength	Confidence Intervals	Weibull Modulus	Confidence Intervals
PSZ Prettau	683.0 ± 70.23 A	700.31 A	678.79-721.90	13.30 A	9.75-17.91
FSZ - Prettau Anterior	438.6 ± 64.1 B	461.27 B	438.61-484.43	8.24 A	6.04-11.10
IPS e.max CAD	248.6 ± 37.3 C	257.21 C	245.53-269.10	8.94 A	6.55-12.04
^a Within the same column, the same letters correspond to statistical similarity. Different letters correspond to statistical difference.					

gentle-beam mode, 2 kV of acceleration voltage, and a gold-sputtered conductive layer.

Fracture Load of Bonded Specimens

Disks of the ceramics (n=30) were obtained as described before, but in different thicknesses (0.5 mm, 1.0 mm, and 1.5 mm). The substrate disks (12 mm diameter × 3 mm) were prepared with Epoxy-resin G10 (Epoxyglas; NEMA Grade FR4, Accurate Plastics Inc, New York, NY, USA), which was selected because it has an elastic modulus similar to that of human dentin.⁴³

The zirconia specimens were airborne particle abraded with 100 µm Al₂O₃ for 15 seconds with an air-abrasion unit Basic Classic (Renfert GmbH, Hilzingen, Baden-Württemberg, Germany) at 0.2 MPa air pressure at a distance of 10 mm from the surface of the specimen at 90° to the abrasion tip. The zirconia and epoxy-resin disks were ultrasonically cleaned in isopropyl alcohol for 5 minutes, followed by 5 minutes in distilled water, and dried with oil-free air, before cementation with RelyX Ultimate Adhesive Resin Cement (3M ESPE, St Paul, MN, USA).

The lithium disilicate specimens were prepared by 10% hydrofluoric acid etching for 20 seconds. Single Bond Universal (3M ESPE) was applied for 20 seconds on lithium disilicate, PSZ, FSZ, and epoxy-resin surfaces. The cement was prepared and applied on the epoxy-resin surface, on which the ceramic

disk was subjected to a load of 10 N for 10 minutes.²⁶ The photoactivation was performed with a light-emitting diode device (Radii Cal; SDI Brasil Industria e Comércio LTDA, São Paulo, Brazil) for 20 seconds at three different sites around the specimen. After 6 minutes, the specimens were stored in distilled water at 37°C for 24 hours.²⁶

The fracture load testing was performed in an MTS 810 universal testing machine. The load was applied by a stainless steel flat punch with a diameter of 1.4 mm at the center of the specimens with the ceramic on the top surface, and the fracture load was recorded in Newtons. Weibull statistics were applied as described for BFS.

RESULTS

BFS and Weibull Analysis

Table 1 presents values of the BFS, characteristic strength and confidence intervals, and Weibull modulus (m) and confidence intervals. The one-way analysis of variance (ANOVA) (F_{2,87} =410.807, p<0.001) was statistically significant, and the Tukey post hoc test showed significant differences among all ceramics. There was a difference among the ceramics in characteristic strength, but there was no difference in the Weibull modulus.

Figure 1 shows the SEM image for lithium disilicate, with its typical needle-like crystal structure. FSZ (Figure 2) showed remarkably larger grains than PSZ (Figure 3).

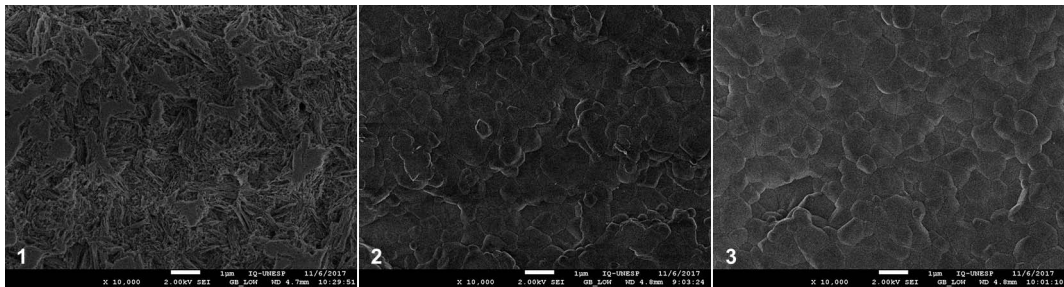


Figure 1. SEM image at 10,000× magnification of lithium disilicate IPS e.max CAD.
Figure 2. SEM image at 10,000× magnification of polycrystalline FSZ Prettau Anterior.
Figure 3. SEM image at 10,000× magnification of polycrystalline PSZ Prettau.

Table 2: Two-way ANOVA Analysis					
Source	Sum of Squares	df	Mean Square	F	p Value
Ceramic	16179006.75	2	8089503.377	54.296	<0.001
Thickness	52160115.90	2	26080057.95	175.048	<0.001
Interaction ceramic thickness	12541238.24	4	3135309.560	21.044	<0.001
Error	38885943.55	261	148988.289		
Total	2068066896	270			
Corrected total	119766304.4	269			
Abbreviations: ANOVA, analysis of variance; df, degrees of freedom.					

Fracture Load and Weibull Analysis of Bonded Specimens

Two-way ANOVA (Table 2) was significant for ceramic type ($p<0.001$), ceramic thickness ($p<0.001$), and interaction between variables ($p<0.001$). Table 3 shows the mean values, standard deviations, and Tukey post hoc comparison. PSZ Prettau had a higher fracture load except with the 0.5 mm thickness, where there was no difference among all ceramics. There was no significant difference between lithium disilicate and FSZ Prettau Anterior ceramic with 0.5 mm and 1.0 mm thicknesses, but at 1.5 mm, lithium disilicate had a higher fracture load.

Weibull analysis results are shown in Table 4. Weibull plots are presented in Figures 4 through 6. The characteristic strength was proportional to the ceramic thickness. For the 0.5 mm thickness, lithium disilicate had a higher value than FSZ. PSZ at 1 mm thickness was more resistant than the other ceramics with the same thickness. There was no significant difference among ceramics with 1.5 mm thickness. Regarding the Weibull modulus, there was no significant difference among all ceramics with the different thicknesses, except that PSZ 0.5 showed the lowest value.

DISCUSSION

The null hypothesis was rejected once significant differences in the BFS among all the materials were

established. The two studied zirconia ceramics are different materials. FSZ - Prettau Anterior has higher content of yttria (8% to 12% mol)²³ which makes it fully stabilized in the tetragonal phase, arising from cubic grains, which improves translucency. PSZ - Prettau has 4–6 mol% of yttria,²³ and hence, it is partially stabilized in the tetragonal phase and is capable of undergoing tetragonal to monoclinic phase transformation toughening.⁹ Thus, PSZ is more resistant than FSZ because the cubic phase is not prone to the phase transformation toughening phenomenon.^{22,23,34} Regarding the lithium disilicate ceramic, lower values were expected due to the glass-ceramic characteristics. Zirconia has a polycrystalline structure, which provides higher strength than vitreous ceramics such as lithium disilicate (Figures 1 through 3). The Weibull modulus (m) is used as a statistical parameter to assess structural reliability, and high “ m ” values correspond to high levels of integrity and structural reliability.³⁷ In the present study, there was no difference among the Weibull moduli of the ceramics in BFS.

Although BFS is an appropriate test to assess the long-term clinical performance of ceramics,^{9,23} it is also important to evaluate the fracture resistance of ceramics bonded on a substrate, as mechanical behavior can be modulated by the modulus of elasticity, cement properties, adhesion to the substrate, and restoration thickness.³⁸

In this study, bonded specimens for fracture load were made using the G10 epoxy resin to simulate a dentin substrate, based on the study of Kelly and others,⁴³ who found no differences in the modulus of elasticity of the G10 epoxy-resin ($E=18.6$ GPa) and dentin ($E=18$ GPa), as well as other studies that used this epoxy resin as a substitute for dentin in mechanical tests.^{27,44-48}

Minimal thickness of a ceramic restoration is important to preserve dental tissues²⁴⁻²⁶ and to allow higher height in the axial wall for short tooth

Table 3: Mean Fracture Strength Values (MPa) for Ceramics With Different Thicknesses Cemented on an Epoxy Substrate ^a			
Thickness, mm	e.max	Prettau Anterior	Prettau
0.5	2271 ± 241 ^{Aa}	2070 ± 275 ^{Aa}	2181 ± 457 ^{Aa}
1.0	2617 ± 346 ^{Ba}	2432 ± 394 ^{Ba}	2863 ± 315 ^{Bb}
1.5	3022 ± 400 ^{Cb}	2741 ± 446 ^{Ca}	3979 ± 515 ^{Cc}
^a Different superscript uppercase letters in columns, and lowercase letters in rows indicate significant difference ($p<0.05$, Tukey test, $n=30$).			

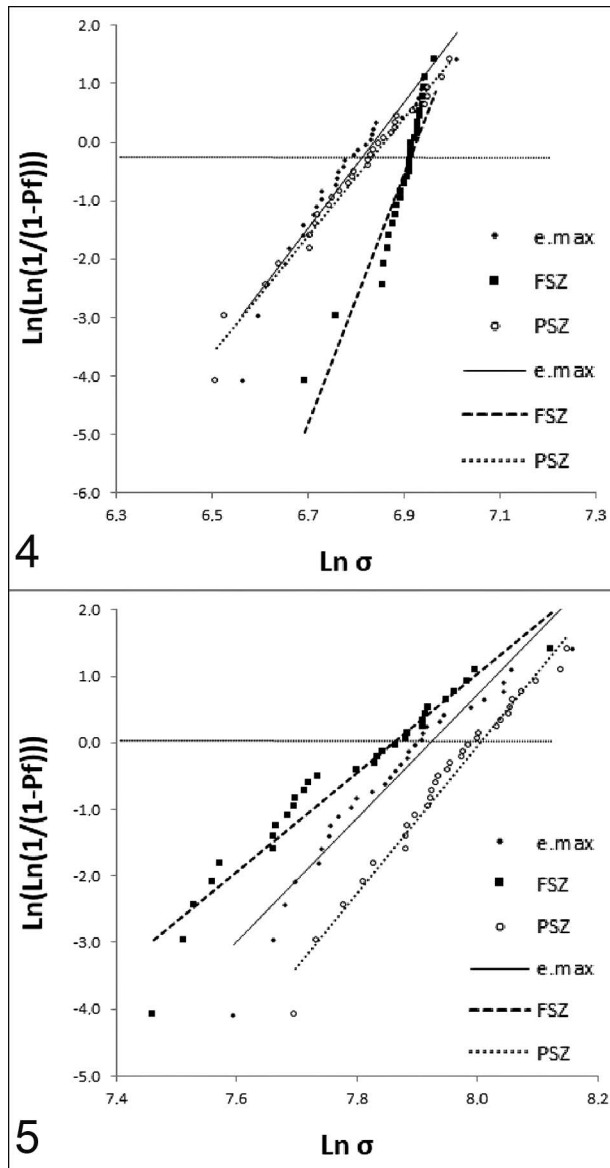


Figure 4. Weibull plots for ceramics in 0.5 mm thickness.
Figure 5. Weibull plots for ceramics in 1 mm thickness.

preparation.²⁷ Recent studies have discussed the influence of thicknesses on the mechanical strength of ceramic crowns.^{24,26-33} Reduction in occlusal thickness decreased the fracture strength,^{27,49} but it is important to determine how thin the restoration can be to maintain clinical effectiveness. Although some studies showed appropriate results at 0.5 mm for translucent monolithic zirconia in molar regions,^{24,26,27,31,33} these studies were conducted with PSZ, which is believed to be stronger. The authors were not able to find studies regarding FSZ.

The null hypothesis for the fracture load of bonded specimens test was also rejected. It was demonstrat-

ed that the resistance of the bonded ceramic was not necessarily related directly to the ceramic strength, as there is no difference in the fracture load for the 0.5-mm-thick ceramic bonded to the epoxy-resin substrate, irrespective of the studied ceramic. Furthermore, lithium disilicate showed fracture loads equivalent or even superior to FSZ for selected thicknesses. Weibull analysis showed that a 0.5-mm-thick ceramic tends to decrease the Weibull modulus, especially for PSZ. Moreover, when esthetics are important, FSZ should not be employed instead of lithium disilicate.

The positive behavior of lithium disilicate, despite its lower BFS, may be related to the more effective adhesion to the substrate.⁵⁰ A resin luting agent can promote a bridging effect on the interfacial surface defects by the effect of volumetric shrinkage of composite resin that causes a tension stress on the cement layer and a compressive stress at the ceramic, thereby strengthening the material and increasing the fracture strength.⁵¹ Lithium disilicate can be etched with 10% hydrofluoric acid to improve surface roughness and wettability, in contrast to zirconia, which is acid-resistant⁵² and has lower adhesion compared with vitreous ceramics. Considering the lower incidences of debonding in the fracture load test in comparison mainly with PSZ, it can be concluded that lithium disilicate has apparently benefited from cementation. A recent study showed that the fracture load of lithium disilicate crowns was directly related to the resin cement properties, whereas a PSZ (Vita YZ T) crown was not affected by the resin and cement; FSZ was not studied.⁵³

The manufacturer of Prettau and Prettau Anterior (Zirkonzahn) indicates a general procedure for cementation but does not recommend any specific adhesive system or cement. However, the manufacturer stresses that the adhesive system and cement instructions must be followed. In this investigation researchers selected the RelyX Ultimate resin adhesive cement that is indicated for zirconia and lithium disilicate ceramics. The cement procedures were followed according to the manufacturer's instructions (3M ESPE), which indicate different pretreatments for zirconia and lithium disilicate. The recommended primer/adhesive, Single Bond Universal, creates a phosphate monomer-based (10-MDP) bond that is capable of bonding to metallic oxides as zirconium oxide.^{54,55} This monomer is present in most zirconia primers,^{56,57} and its application over an airborne particle-abraded zirconia surface has led to effective adhesion.^{54,58} In

Table 4: Weibull Modulus and Characteristic Strength With a Confidence Interval of 95% for the Materials ^a				
Material, mm	Characteristic Strength, N	Confidence Intervals	Weibull Modulus, m	Confidence Intervals
LD 0.5	2449.42 C	2333.30-2567.88	8.55 A,B	6.27-11.51
LD 1.0	2675.51 B	2578.55-2773.29	11.25 A	8.25-15.15
LD 1.5	2893.98 A	2790.08-2998.72	11.36 A	8.33-15.29
FSZ 0.5	2134.43 D	2050.04-2219.82	10.29 A	7.55-13.86
FSZ 1.0	2558.14 B,C	2422.74-2697.06	7.64 A	5.60-10.28
FSZ 1.5	2781.43 A,B	2673.57-2890.48	10.50 A	7.70-14.14
PSZ 0.5	2341.67 C,D	2173.76-2517.37	5.58 B	4.09-7.51
PSZ 1.0	2956.26 A	2853.19-3060.06	11.70 A	8.58-15.76
PSZ 1.5	2781.43 A,B	2675.57-2890.48	10.50 A	7.70-14.14

Abbreviations: LD, lithium disilicate; FSZ, fully stabilized zirconia; PSZ, partially stabilized zirconia.
^a Within the same column, the same letters correspond to statistical similarity. Different letters correspond to statistical difference.

addition to 10-MDP monomer, Single Bond Universal has silane, which is important for adhesion to the lithium disilicate glass ceramic.

An issue to be considered is the dependence of the self-cure mechanism on the amount of blue light passing through the ceramics in dual-cure luting cement polymerization.⁵⁹ Thickness and translucency of the ceramic layer, curing light unit intensity, and exposure time affect dual-cure cement polymerization. Considering that zirconia ceramics are less translucent than glass ceramic, lower transmitted irradiance in zirconia is expected compared with lithium disilicate. Moreover, differences among optical properties of monolithic zirconia should be considered. Sulaiman and others⁴¹ studied the light irradiance of monolithic zirconia ceramics at variable thicknesses and observed that the FSZ Prettau

Anterior allowed significantly higher values of light irradiance than the PSZ Prettau, but the energy transmitted through ceramics decreased as the layer thickness increased, irrespective of ceramic brand.

Another issue is that accelerated artificial aging was not evaluated in this study. The effect of aging in different properties of PSZ ceramics has been studied by several authors who discussed methods and mechanisms of aging and showed that tetragonal to monoclinic transformation can be induced by steam autoclave to produce hydrothermal degradation, which is believed to be similar to the low temperature degradation. The effect of hydrothermal degradation depends on the zirconia ceramic composition, grain size, stress conditions, and primarily on the steam autoclave temperature and running time. Mechanical properties decreasing on PSZ is evident only in more severe protocols. However, the correlation of artificial aging methods and its effective correspondence with clinical performance of zirconia is controversial.⁶⁰ Moreover, FSZ is not affected by artificial aging once supersaturation by yttria increases the amount of cubic phase and provides more stability to the tetragonal phase.^{34,61}

It is important to highlight limitations of this *in vitro* study. The fracture load test was performed in simple geometry specimens with a short-term storage and limited to a particular luting system. Even at 0.5 mm thickness, irrespective of the ceramic material, the fracture loads were higher than 1000 N, which is higher than those required to ensure proper clinical performance.⁶² Moreover, the shape of the actuator used in the fracture load of bonded specimens produces contact surface damage with much cone cracking, which does not simulate clinical failure that is typically produced by subsurface radial cracking.⁴³ Further studies, including differ-

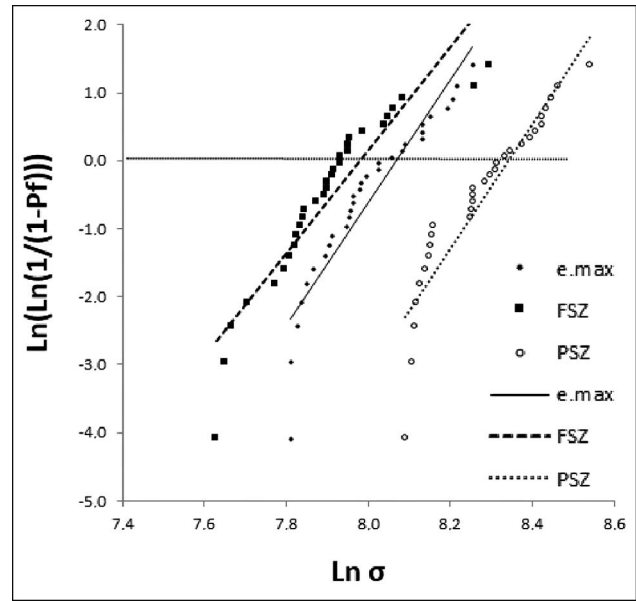


Figure 6. Weibull plots for ceramics in 1.5 mm thickness.

ent cementation protocols, aging challenges, and fatigue testing, could ensure a more realistic approach to the clinical conditions.

In summary, the performance of ceramic materials bonded to a substrate does not seem to rely solely on the mechanical properties of the ceramics but may be governed by a complex scenario consisting of several variables, such as mechanical properties of ceramic and substrate, bonding efficacy of resin cement, ceramic translucency, quality of curing light, and effective self-cure activation, among others factors.

CONCLUSIONS

With the limitations of this study, it was concluded that despite the higher BFS values of PSZ and FSZ compared with lithium disilicate, there was no difference among the ceramics in the fracture load in bonded specimens at 0.5 mm thickness. Moreover, fracture load of bonded lithium disilicate was equivalent or superior to that of FSZ at the same thickness. Regarding reliability, there was no difference in the Weibull modulus of the ceramics in the BFS test or in the fracture load of bonded specimens.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Araraquara Dental School, Univ Estadual Paulista – UNESP in Brazil.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Effect of Thermocycling on Biaxial Flexural Strength of CAD/CAM, Bulk Fill, and Conventional Resin Composite Materials

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Clinical Relevance

Biaxial flexural strength of tested materials vary with composition and thermocycling significantly decreases their strength. Future clinical studies should evaluate if recommendations based on cavity size and tooth location improve long-term performance.

SUMMARY

New resin-based restorative materials have been developed, such as computer-aided design/computer-aided manufacturing (CAD/CAM) and bulk-fill composites, as an alternative to traditional layering techniques. This study evaluated the biaxial flexural strength (BFS) before and after thermocycling of five different resin composites: one hybrid resin/

ceramic CAD/CAM indirect material, Lava Ultimate CAD-CAM Restorative (LU, 3M Oral Care); a conventional composite, Filtek Z350 XT (Z350, 3M Oral Care); two bulk-fill composites, Tetric N-Ceram Bulk Fill (TBF, Ivoclar Vivadent) and Filtek Bulk Fill (FBF, 3M Oral Care); and one bulk-fill flow resin composite, Filtek Bulk Fill Flow (FBFF, 3M Oral Care). Three hundred disc-shaped specimens (6.5 mm

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in diameter and 0.5 mm thick) were fabricated and divided into five groups ($n=30$ for each composite and condition). The BFS test was performed in a universal testing machine at a crosshead speed of 0.5 mm/min immediately (i, 24 hours) and after thermocycling (a, 500 thermal cycles of 5°C to 55°C with a 30-second dwell time). The Weibull modulus (m) and characteristic stress (η) were calculated, and a contour plot was used (m vs η) to detect differences between groups (95% two-sided confidence intervals). Significantly higher characteristic stress was observed for LUi (286.6 MPa) and Z350i (248.8 MPa) compared to the bulk-fill groups (FBFi=187.9 MPa, FBFFi=175.9 MPa, TBFi=149.9 MPa), with no differences between LUi and Z350i. Thermocycling significantly decreased the characteristic stress of all groups with the highest values observed for LUa (186.7 MPa) and Z350a (188.9 MPa) and the lowest for FBFFa (90.3 MPa). Intermediate values were observed for FBFa (151.6 MPa) and TBFa (122.8 MPa). The Weibull modulus decreased only for FBFa compared to FBFi. Composition and thermocycling significantly influenced the biaxial flexural strength of resin composite materials.

INTRODUCTION

Although excellent esthetic results can be obtained with direct composite resin restorations, this material still presents disadvantages, such as polymerization shrinkage stress and dentin adhesion, which may lead to marginal infiltration, postoperative sensitivity, and a decrease in dentin bond strength.^{1,2} To meet the recommendations of the Minamata Convention, recent developments in composite resins include different compositions to minimize polymerization contraction stress and different fabrication methods, including milling from computer-aided design/computer-aided manufacturing (CAD/CAM) resin composite blocks or additive manufacturing, such as 3D printing.³⁻⁵

Despite reported high survival rates of composite resin restorations when compared to amalgam, even in wide restorations,⁶ there is still a need for improvement of this material because it undergoes loss of mechanical properties and esthetics as a function of fatigue, aging at body temperature, and a moist environment.⁷ The cause of discoloration, polish loss, opacity increase, and loss of adhesion, as well as the appearance of fractures, is multifactorial but highly influenced by internal chemical

changes in the material that occur due to degradation, which can be simulated by artificial aging methods.⁸

The hydrothermal degradation of resin composites is mediated by sorption and solubility, phenomena that produce chemical changes with deleterious effects on the mechanical properties of polymeric materials.⁹ The diffusion of solvents into the polymer network leads to a volumetric expansion due to the separation of polymeric chains and depends on the composition and microstructure of the materials.¹⁰ Swelling caused by absorption of aqueous solvents is accompanied by a loss of non-reacted components,¹¹ erosion of the filler-matrix interface, and plasticization with a reduction in hardness, stiffness, wear resistance, and flexural strength,^{9,12} which may compromise long-term clinical results of composite resin materials.

Although there are several laboratory tests available for dental composite resins, they present a challenge in simulating clinical performance. However, they can guide understanding the effects of changes in composition or processing on material properties. In flexural strength testing, flexural forces are generated to simulate clinical situations where materials need to withstand flexing, especially in the posterior region. Although not confirmed clinically, high flexural strength is desired for these materials that might experience cracking under occlusal stress.¹³

Also, because of the heterogeneous composition of resin composites and the dissimilar coefficient of thermal expansion of their components, thermal stresses are easily generated in the material and may be exacerbated by thermal cycling.¹⁴ Thus, thermocycling has been widely accepted in the literature as a method to promote the degradation of the mechanical properties¹⁵ and the bond strength of resin composite materials.¹⁶⁻¹⁸

A promising approach to overcome the difficulties of sensitive and time-consuming restorative procedures using the incremental technique include low-shrinkage composite resins that allow the clinician to apply layers up to 5 mm thick.¹⁸ Bulk placement of resin composites has been reported to provide more compact fillings, preventing void contamination between composite layers.^{17,19}

Another alternative to direct restoration techniques is milling restorations from composite blocks in CAD/CAM systems. The main advantages include production of homogeneous blocks with a high degree of conversion, reduced equipment wear

Table 1: *Manufacturers, Composition, and Batch Numbers of the Tested Materials*

Brand Name	Type	Manufacturer (Lot Number)	Monomers	Filler Type	Filler Volume (%)	Batch
Lava Ultimate (LU)	Resin/ceramic hybrid material for CAD/CAM	3M Oral Care (St Paul, MN, USA)	UDMA	Silica, zirconia, nanoparticle clusters	80	550835
Filtek Z350 XT (Z550XT)	Nanoparticle	3M Oral Care	UDMA, bis-GMA, bis-EMA, TEGDMA	Silica, zirconia, clusters, zirconia/silica aggregated particles	78.5	1524600312
Filtek Bulk Fill (FBF)	Bulk fill	3M Oral Care	Bis-GMA, bis-EMA, UDMA, TEGDMA, Procrylat resins	Zirconia/silica, ytterbium trifluoride	76.5	1522200095
Tetric N-Ceram Bulk Fill (TBF)	Bulk fill	Ivoclar Vivadent (Schaan, Liechtenstein)	Bis-GMA, UDMA	Ba-Al-Si glass, prepolymerized filler (monomer, glass filler, and ytterbium fluoride), pherical mixed oxide	61% of inorganic fillers and 17% prepolymerized "isofillers"	T40644
Filtek Bulk Fill Flowable Composite (FBFF)	Flowable bulk fill	3M Oral Care	Bis-GMA, UDMA, bis-EMA, Procrylat resins	Zirconia/silica, ytterbium trifluoride	64.5	1526900194

Abbreviations: UDMA, urethane dimethacrylate; bis-GMA, bisphenol-A glycidyl methacrylate; bis-EMA, ethoxylated Bisphenol A glycidyl methacrylate; TEGDMA, triethylene glycol dimethacrylate.

(CAD/CAM burs), reduced cost of fabrication, and ease of adjustments and repair.²⁰⁻²² The ceramic particles embedded in the organic matrix can be of various compositions and, due to the high-filling percent content, these materials have been included in the American Dental Association's Code of Dental Procedures and Nomenclatures as ceramic/porcelain. They are classified elsewhere as hybrid or ceramic-like materials (resin-matrix ceramics) due to the presence of an organic matrix, which excludes them from ceramics as known in materials science.²³ Most of them have been indicated for inlays, onlays, veneers, and crowns.

Because of the presence of an organic matrix, resin-based composite materials are subject to aging, which can decrease mechanical properties and clinical longevity. Thus, in the present study, we evaluated the biaxial flexural strength (BFS) and the effect of aging by thermocycling on a group of resin-based restorative materials, including conventional composites, CAD/CAM, and bulk-fill composite resins. The hypotheses were 1) that composition and manufacturing method would influence the characteristic stress or Weibull modulus of composite resins and 2) that the characteristic stress of composite resins would decrease after aging regardless of composition or fabrication/layering method.

METHODS AND MATERIALS

Specimen Preparation

Three hundred composite resin disc-shaped specimens (6.5 mm in diameter and 0.5 mm thick)²⁴

were fabricated using a Teflon mold and divided into five groups (n=60 for each composite) as follows: 1) resin nanoceramic - Lava Ultimate CAD-CAM Restorative (LU, 3M Oral Care, 3M, St Paul, MN, USA), 2) resin nanoceramic - Filtek Z350 XT (Z350, 3M Oral Care), 3) bulk fill composite - Tetric N-Ceram Bulk Fill (TBF, Ivoclar Vivadent, Schaan, Liechtenstein), 4) bulk-fill composite - Filtek Bulk Fill (FBF, 3M Oral Care), and 5) bulk-fill flow resin composite - Filtek Bulk Fill Flow (FBFF, 3M Oral Care). Details regarding manufacturers, composition, and batch of the composites used are detailed in Table 1. Half of the samples (n=30) for each group were tested for BFS immediately (i, 24 hours) after fabrication and the other half (n=30) after thermocycling (a).

For the resin nanoceramic, bulk-fill, and bulk-fill flow composite resins, a single increment of composite material was confined in the Teflon mold between two opposing polyester strips. Light curing was performed for 20 seconds on both sides with an LED unit (Valo, Ultradent, South Jordan, UT, USA) having an irradiance of 1000 mW/cm². The specimens were then removed from the mold and excesses were eliminated with a scalpel blade (Surgical Scalpel Blade No. 15, Swann-Morton, Sheffield, England). The CAD-CAM Lava Ultimate blocks were milled into 6.5-mm-diameter cylinders using Cerec (Dentsply Sirona Inc, Bensheim, Germany) and then sliced using a slow-speed diamond saw (Extex Corp, Enfield, CT, USA) in a precision water-cooled machine (Isomet Low Speed Saw,

Table 2: Results of Biaxial Flexural Strength Test^a

Group	Immediate		Aged	
	Characteristic Stress, MPa (95% CB)	Weibull Modulus (<i>m</i>)	Characteristic Stress, MPa (95% CB)	Weibull Modulus (<i>m</i>)
Lava Ultimate (LU)	286.6 (307.3-267.2) Aa	5.1 (6.7-3.9) Aa	186.7 (195.5-178.3) Ba	7.6 (10-5.81) Aa
Filtek Z350 XT (Z350)	248.8 (266.5-232.2) Aa	5.2 (6.8-4) Aa	188.9 (188.9-178.3) Ba	8.1 (10.6-6.2) Aa
Filtek Bulk Fill (FBF)	187.9 (194.9-181.1) Ab	9.9 (12.9-7.6) Ab	151.6 (169.2-135.9) Bb	3.1 (4.1-2.4) Bb
Tetric N-Ceram Bulk Fill (TBF)	149.9 (157.5-142.8) Ac	6.7 (8.6-5.3) Aab	122.8 (127.8-118.0) Bc	9.0 (11.7-7.0) Aa
Filtek Bulk Fill Flow (FBFF)	175.9 (192.5-160.8) Ab	4.2 (5.3-3.2) Aa	90.3 (103.2-79.0) Bd	2.5 (3.4-1.9) Ab

^a Uppercase letters show differences between rows (comparing immediate and aged groups), and lowercase letters show differences between columns (comparing materials). Values in parentheses represent 95% upper and lower confidence bounds.

Buehler, Lake Bluff, IL, USA) to obtain 60 disc-shaped specimens approximately 0.5 mm thick. All specimens were manually polished under water cooling with 800-, 1200-, and 2000-grit silicone carbide paper (Carbimet Paper Discs, Buehler), and their dimensions were measured using a digital caliper (Mitutoyo, Takatsu-ku, Kanagawa, Japan). Although clinical protocols may be different, the standardization of the polishing protocol during laboratory testing allowed for comparison of the characteristic stress and Weibull modulus between all groups.

The specimens were dark stored dry at 37°C for 24 hours (i). One-half of the specimens for each composite resin (n=30) were subjected to thermocycling of 5°C to 55°C with a 30-second dwell time at each temperature, and 500 thermal cycles (a).

Biaxial Flexural Strength

A piston-on-ring device was used for the biaxial flexural test, following methodology described by Rueggeberg and others.²⁴ Each disc was placed into a custom-made jig with 1-mm circumferential support, and the load was applied at the center of the specimen using a flat piston with a 0.5-mm diameter. The device was positioned in a universal testing machine (Kratos, São Paulo, Brazil), and specimens were tested at a crosshead speed of 0.5 mm/min until failure. The maximum load was recorded for each specimen, and the following formulas were used to calculate biaxial flexural strength:

$$S = -0.2387P(X - Y)/d^2$$

$$X = (1 + \nu)\ln(r_2/r_3)^2 + ([1 - \nu]/2)(r_2/r_3)^2$$

$$Y = (1 + \nu)(1 + \ln[r_1/r_3]^2) + (1 - \nu)(r_1/r_3)^2$$

where S = biaxial flexural strength (MPa), P = fracture load (N), d = disc specimen thickness at fracture site (0.5 mm), ν = Poisson ratio (0.25), r_1 = radius of support circle (2.75 mm), r_2 = radius of loaded area (0.25 mm), and r_3 = radius of the specimen (3.25 mm).

The fractured specimens were examined with an Axio Zoom V16 Stereo Zoom Microscope (Carl Zeiss, Oberkochen, Germany).

Statistical Analysis

Strength data (MPa) were analyzed using the Weibull distribution as an alternative to the normal distribution due to its ability to evaluate the properties, characteristic stress (η), and Weibull modulus (m) of the materials, and a contour plot was used (m vs η) to detect differences between groups (95%, two-sided confidence intervals).

RESULTS

Table 2 shows the results for the characteristic stress (MPa) and the Weibull modulus (m) for the immediate and aged groups with respective upper and lower 95% confidence bounds. Significantly higher characteristic stress was observed for LU_i (286.57 MPa) and Z350_i (248.76 MPa) than all bulk-fill composites (FBF_i=187.88 MPa, FBFF_i=175.93 MPa, TBF_i=149.93 MPa), with no differences between LU_i and Z350_i. TBF_i presented the lowest characteristic stress (149.93 MPa) among all immediately tested groups.

The characteristic stress of all groups significantly decreased after thermocycling. Among aged groups, the highest values were observed for LU_a (186.71 MPa) and Z350_a (188.94 MPa) and the lowest for FBFF_a (90.34 MPa). Intermediate values were observed for FBF_a (151.6 MPa) and TBF_a (122.77 MPa). The Weibull modulus was not significantly

different for the same material tested immediately and after aging, except for FBFa, which showed a significantly lower Weibull modulus after aging ($m=3.12$).

The information regarding characteristic stress vs the Weibull modulus is instructively presented in a contour plot (Figure 1A), where differences between groups (95%, two-sided confidence intervals) are detected when an overlap does not exist between the contours. When plotting the probability of survival as a function of characteristic stress (Figure 1B), a clear trend in decrease of survival was observed for all aged groups. Also, the same differences between groups observed in the contour plot regarding characteristic stress were observed for probability of survival between groups.

Fractographic analyses showed different fracture patterns for the LU and Z350 groups when compared with bulk-fill and bulk-fill flow resin composites. LU and Z350 composites fractured in several pieces (three to five), while bulk fill composites fractured in fewer pieces (two or three). Fractographic analysis showed that regardless of aging or group, fractures always initiated on the tensile side, leaving marks such as hackles which depicted the direction of crack propagation, and a surface flaw as the fracture origin (Figure 2).

DISCUSSION

Composite resins have been widely used as a more esthetic, mercury-free alternative for amalgam restorations. Although clinical research data have shown high survival rates for resin composite restorations, secondary caries and bulk fractures are still the main reasons for clinical failure.²⁵ Both issues are commonly associated with sensitive restorative techniques, polymerization shrinkage, and hydrothermal degradation.^{9,26}

In order to overcome such problems, efforts in polymer chemistry engineering have led to the development of bulk-fill resin composite materials with reduced volumetric shrinkage and increased depth of cure that allows the clinician to place increments up to 4 or 5 mm thick.²⁷ Considering that degree of conversion and material composition are determinants of the mechanical properties of resin composites, modifications in their composition to tailor polymerization shrinkage are challenging when fracture strength needs to be maintained or improved.

The enhanced translucency of bulk-fill resin composites allows greater light transmission and

deeper polymerization in greater increments. Further, modification in the composition of resin composites, such as the addition of stress-relieving monomers, different and more reactive photoinitiators, and the incorporation of specific types of fillers, such as prepolymerized particles and glass fibers, allows modulation of the polymerization reaction.²⁸ Nevertheless, the introduction of prepolymerized particles as inorganic fillers may result in a decrease in mechanical properties due to a relatively low ceramic particle volume.

Among tested groups, TBF presented the lowest ceramic filler content (approximately 61% [vol] and 17% for prepolymerized polymer fillers or “isofillers”), which may explain its lowest characteristic stress among all immediately tested groups. The results of BFS testing showed a direct correlation between inorganic content and BFS for all immediately tested materials, which led us to accept our first hypothesis that composition and manufacture method would influence the characteristic stress and Weibull modulus of composite resins. Two composites containing a high content of ceramic particles (zirconia and silica), regardless of fabrication method (layered Z350 or milled LU), presented the highest characteristic stress among all groups. With lower filler content, intermediate characteristic stress values were observed for FBF and FBFF with no statistical difference between them. In contrast, a significantly higher Weibull modulus was observed for FBF than FBFF, likely due to filler volume differences (76.5% for FBF and 64.5% for FBFF) that eventually led to a higher flaw population in the flowable bulk-fill composite.

Among a great variety of factors, mechanical properties of composite resins depend on their composition and manufacture. Highly inorganic filled composite resins have been associated with improved flexural strength²⁹ and Young's modulus,³⁰ while a greater organic content has been commonly associated with increased hydrothermal degradation and greater polymerization shrinkage. In addition, defects and porosities may work as stress raisers, frequently associated with failure origin, as shown in our fractographic analysis. Within this context, CAD/CAM resin-based composite materials have been developed as an alternative to conventional layering of composite resins for the manufacture of indirect restorations through the machining of homogeneous CAD/CAM blocks. Although higher characteristic stress and Weibull modulus were expected for CAD/CAM blocks, no differences were observed between layered and

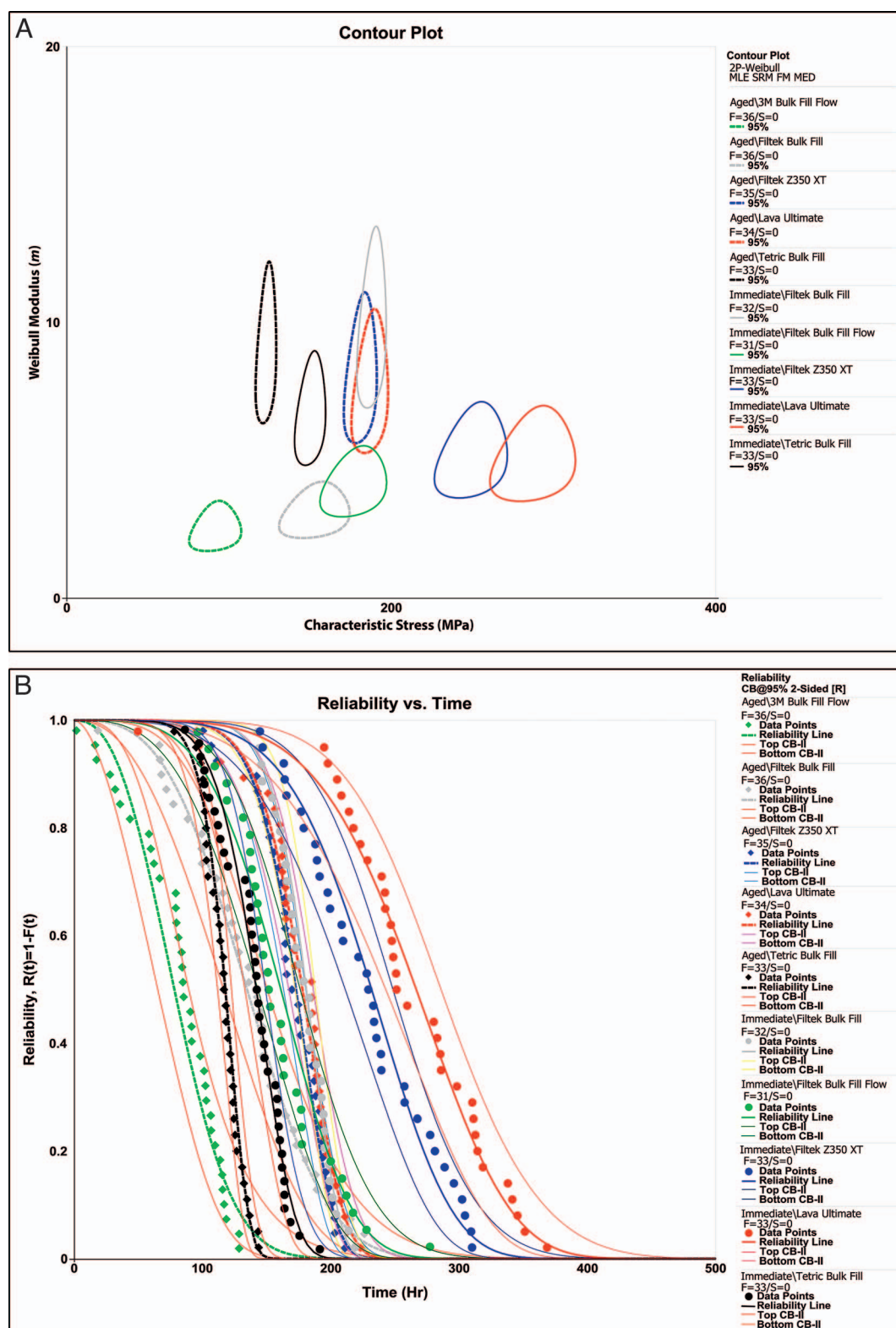


Figure 1. (A): Contour plot of characteristic strength (MPa) vs Weibull modulus (m). Dotted contour lines indicate aged samples. (B): Probability of survival vs characteristic strength shows a significant decrease in survival for all aged composites. Lowest survival was observed for FBFFa, intermediate values for FBFa and TBFa, and significantly higher values for LUa and Z350a (non-overlap between upper and lower confidence intervals shown by the red lines).

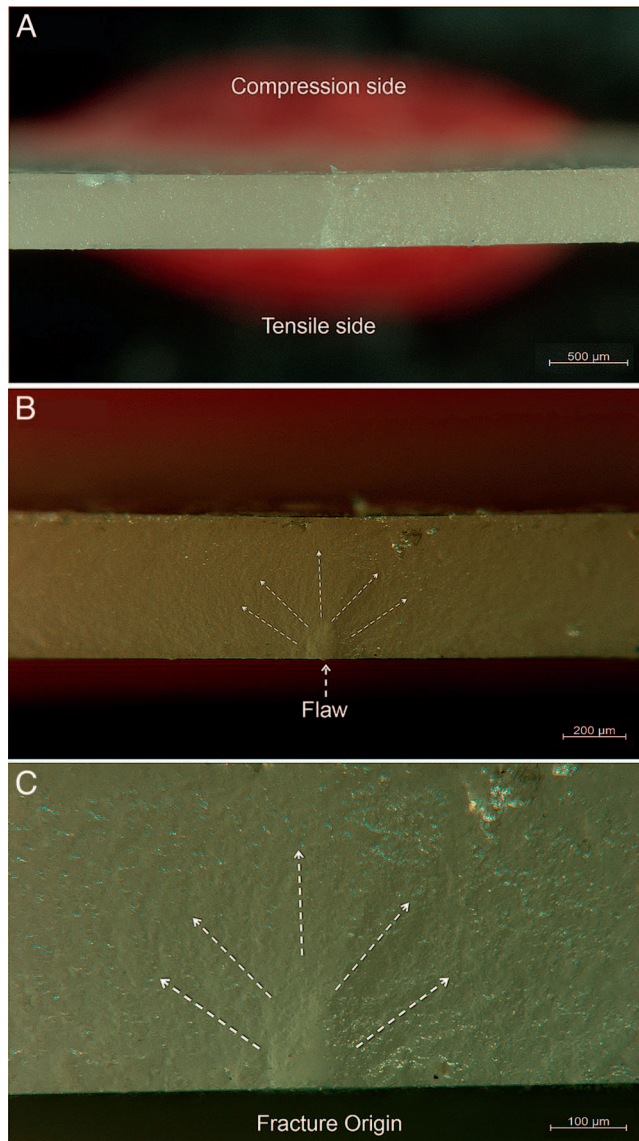


Figure 2. (A): Representative sample fractured in biaxial flexural strength showing compressive and tensile sides under low magnification. (B): Higher magnification at the bottom center of the specimen shows hackles that allow trace back to the failure origin shown in higher magnification (C).

milled resin “nanoceramics” tested immediately. Also, thermocycling reduced these parameters in the two composite resins similarly, suggesting that flaw population differences were not great enough to affect their characteristic stress and Weibull modulus. Thus, our second hypothesis was also accepted.

The present study used 500 thermal cycles, an amount that has been suggested to represent approximately six months of *in vivo* usage.^{16,31} Other authors consider that 10 thermal cycles represent one day of use.³² However, the correlation between

the number of thermal cycles and clinical service is still debated with limited conclusive evidence between the *in vitro* simulation and *in vivo* aging of resin composites.¹⁶ Regardless of the protocol used, thermocycling protocols have been reported to be efficient in promoting degradation of the mechanical properties of resin composites^{15,33} as observed in this study.

Among several options for flexural testing described in the materials literature, a piston-on-ring test was used in the present study, as have others,^{24,28} to determine the biaxial flexural strength. The main advantage of this test consists in the concentration of the maximum tensile stresses in the central loading area where the failure is initiated, leading to an accurate measurement of the load necessary to fracture the specimen (further plotted in strength) and the elimination of spurious edge failures frequently seen in three-point flexure testing.³⁴

The Weibull distribution was selected for the statistical analysis due to its ability to evaluate the properties, characteristic stress, and Weibull modulus. Mean strength values associated with standard deviations have been frequently used to report composite resin strength data;³⁴ however, the lack of homogeneity in the distribution of the flaws from specimen to specimen may result in failures at lower stresses.³⁵

The Weibull modulus (m) measures the variability of the results, where higher m values indicate greater structural reliability (higher homogeneity), whereas lower m values reflect more flaws in the structure (lower homogeneity) and thus a decrease in reliability. The clinical translation is that a resin composite with a higher Weibull modulus denotes a more homogeneous distribution of the flaws and a more reliable clinical performance (failure in a narrower range of stress). When BFS values are evaluated as characteristic stress, the data indicate the load at which 63.2% of the specimens of each group would fail.^{36,37}

Success of composite resin restorations is multifactorial and several variables are involved.³⁸ There are clinical-, patient-, professional-, and material-related factors that may influence the survival of dental restorations.³⁹ Understanding of flexural strength ranking is a starting point in material selection, along with clinical indications and patient factors.³⁸ Clinical studies are warranted to determine the survival of different restorative materials under controlled scenarios.

CONCLUSIONS

Composition and thermocycling significantly influenced the BFS of resin composite materials. Future clinical studies should evaluate if specific recommendations based on cavity size and tooth location should be made to improve their long-term performance.

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Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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