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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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In Memoriam

Maxwell H. "Max" Anderson DDS, MS, MEd

September 13, 1943–October 28, 2013

Operative Dentistry lost a timeless member and to so many of us a trusted and dear friend with the passing of Dr. Max Anderson on October 28, 2013 at his home in Sequim, Washington. Max remained a warrior to the end of his four year battle with cancer.

Max was born in Lincoln, Nebraska, during the height of World War II. He never had a chance to know his father as his father died while deployed to the far east while serving to "fly the hump" into China. I don't believe he could have been given a more appropriate name as he never did anything less than "to the max".

Max's own service to our nation began with his enlistment into the Navy in 1963. Max served in submarines and was a qualified nuclear reactor operator for four years aboard USS Roosevelt (SSBN 600). It was while serving in Groton, CT, in 1968 that he met his life-long partner in Suzanne Fontaine. They were married in 1968.

Max left the Navy in 1970 as an IC1 to return to college and pursue his desire to become a dentist. While in Lincoln at the dental school, he became close friends with his classmate Bob Garcia. Bob proceeded to introduce Max to his father-in-law, Dr. Bill Ferguson and Max was now on his road to dedicate himself to operative dentistry. He graduated from the University of Nebraska, School of Dentistry in 1976 and was commissioned in the Navy as a General Practice Resident at Portsmouth Naval Hospital. He was then assigned to the USS Raleigh (LPD-1) for two years. Following a tour at NAS Brunswick, Maine, Max was selected to attend the University of Michigan for a Master's degree in Operative Dentistry. Again, under the watchful eyes of Drs. Charlie Cartwright, Walter Loesche, and many others, Max was further directed toward operative dentistry, particularly the interaction with the caries process. He developed a lifelong friendship with his classmates Bill Gregory, Mike Molvar, Mark Fitzgerald and me. Tours at NDC San Diego, where many AEGD's fell under his spell, to his final tour on the faculty at the Naval Dental School, Bethesda, showed his deep dedication and devotion to superb dental care emphasizing both restorative and preventive strategies to enhance the oral health of our sailors and Marines. While at Bethesda, Max served as the Specialty Leader for Operative Dentistry to the Navy Surgeon General.

Dr. Max Anderson

Max retired from the Navy in 1990 to become a member of the dental faculty at the University of Washington. During this time Max also served as the Editor of the Journal of Operative Dentistry for three years and oversaw several expansions in its format. He was one of the initial Associate Editors of the Journal of Evidence-Based Dental Practice. He served on the Executive Council of the Academy of Operative Dentistry.

In 1994, and for the next 10 years, Max was Vice President and Dental Director of Washington Dental Service. While there he developed the largest dental data warehouse then in existence to provide information to all Delta Dental companies and to various dental specialty groups.

In 2005, Max cofounded C3Jian, Inc. with Dr. Wenyuan Shi of UCLA with the initial goal of developing a cure for dental caries using a strategy of targeting specific pathogens. Max had more than 60 articles published in dental literature and provided his unique presentation skills to groups around the world.

Max was presented with the Hollenback Award for excellence in Operative Dentistry in 2012.

Max was innovative, devoted, determined and dedicated to each and every endeavor. He was ever available to his residents and his colleagues and always valued his time in support of oral health.

In the tradition of the Navy there is a poem titled "The Watch" and its conclusion certainly describes Max.

"Shipmate, the watch stands relieved,
Relieved by those you have led, trained and guided.
Shipmate you stand relieved, we have the watch.
Boatswain. . .Standby to pipe the side
Shipmate Anderson's going ashore"

Max is survived by his wife of 45 years, Suzanne, son Erik and daughter Morgan Nicole.

Donations can be made to: Assured Hospice of Clallam & Jefferson Counties of Washington; Sequim Food Bank; Moving Toward Independence, Napa, CA, or to the Founder's Fund of the Academy of Operative Dentistry.

Submitted by Ronald C. House, DDS, MS

Editorial

New Blood

Jeffrey A. Platt, D.D.S., M.S., Editor

A gifted researcher and teacher that I once knew carried with him a significant fault – something that is a struggle for many of us. As I was holding a new grandchild over the recent holidays, I was reminded of the importance of overcoming this fault. You see, this previous mentor of mine struggled with passing on the reigns to bright minds who could replace him. Don't we all have some desire to hang on to our position in life, to not let go? But, we must pass on what we have or what we have may perish.

For example, are you passing on the importance of membership in dental academies — organizations that continue to focus on the excellence of care that many believe our profession should embody? If you are an academy member, are you willing to actively pursue a new member — someone who demonstrates a desire to continue the legacy of excellence? OR, are you waiting for someone to ask you if he or she could be invited to participate in your academy?

Journal editor is a role that must be passed on someday. This is the last year of my first term as editor, an honor for which I am very grateful. However, it is rare for someone to continue beyond two terms. I ask myself: Who is willing to make the commitment needed to pursue this role after me? Often, the next in line comes from the ranks of reviewers and associate editors. So, I am asking you: if you have a desire to take on the role of editor at some point in the future, let me know of that desire. Although I cannot promise that it will ever happen, I greatly desire to provide the opportunities necessary for bright, new blood to carry on the mission of excellence.

Nurture new blood. Encourage new blood. Do not be afraid of new blood. For it is through new blood that legacies develop, continue and flourish.

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Clinical Research

A New Universal Simplified Adhesive: 18-Month Clinical Evaluation

J Perdigão • C Kose • AP Mena-Serrano
EA De Paula • LY Tay • A Reis
AD Loguercio

Clinical Relevance

At 18 months, the new multimode adhesive, Scotchbond Universal Adhesive, fulfilled the American Dental Association criteria required for full approval. Its clinical behavior is reliable when used in noncarious cervical lesions and may not depend on the bonding strategy.

SUMMARY

Purpose: To evaluate the 18-month clinical performance of a multimode adhesive (Scotchbond Universal Adhesive, SU, 3M ESPE, St Paul, MN, USA) in noncarious cervical lesions (NCCLs) using two evaluation criteria.

Materials and Methods: Thirty-nine patients participated in this study. Two-hundred restorations were assigned to four groups: ER*m*, etch-and-rinse + moist dentin; ER*d*, etch-and-rinse + dry dentin; S*et*, selective enamel etching; and SE, self-etch. The composite resin, Filtek Supreme Ultra (3M ESPE), was placed incrementally. The restorations were evaluated at baseline, and at 18 months, using both the World Dental Federation (FDI) and the United States Public Health Service (USPHS) criteria. Statistical analyses were performed using Friedman repeated-measures analysis of variance by rank and McNemar test for significance in each pair (α=0.05).

Results: Five restorations (SE: 3; S*et*: 1; and ER*m*: 1) were lost after 18 months ($p>0.05$ for either criteria). Marginal staining occurred in four and 10% of the restorations evaluated ($p>0.05$), respectively, for USPHS and FDI criteria. Nine restorations were scored as

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***bravo* for marginal adaptation using the USPHS criteria and 38%, 40%, 36%, and 44% for groups ER*m*, ER*d*, S*et*, and SE, respectively, when the FDI criteria were applied ($p>0.05$). However, when semiquantitative scores (or SQUACE) for marginal adaptation were used, SE resulted in a significantly greater number of restorations, with more than 30% of the total length of the interface showing marginal discrepancy (28%) in comparison with the other groups (8%, 6%, and 8%, respectively, for ER*m*, ER*d*, and S*et*).**

Conclusions: The clinical retention of the multimode adhesive at 18 months does not depend on the bonding strategy. The only differences between strategies were found for the parameter marginal adaptation, for which the FDI criteria were more sensitive than the USPHS criteria.

INTRODUCTION

The constant development of dental adhesive materials over the past decades has resulted in the launching of adhesives without reliable clinical validation. Often, a new version of a dental adhesive is introduced to the dental market when clinical studies with the previous version are still being carried out. This is increasingly common, especially with one-step self-etch adhesives. Ideally, once an adhesive is tested *in vitro*, a clinical trial must follow immediately to evaluate the clinical effectiveness of the tested adhesive.

Bonding to enamel is based primarily on micromechanical interlocking of resin monomers into the enamel microporosities created by chemical dissolution of hydroxyapatite crystallites using phosphoric acid.[1,2] For most dental adhesives, the depth of the etching pattern plays a significant role in the magnitude of the enamel bond strengths.[3] Phosphoric acid etching significantly increases the bond strength of one-step self-etch adhesives to enamel.[4,5] However, when enamel is ground or beveled, self-etch adhesives tend to result in enamel bond strengths comparable with those of etch-and-rinse adhesives.[6,7] In class I and class II composite restorations, phosphoric acid–etched margins resulted in tighter marginal integrity and a lower degree of discoloration.[8]

Because self-etch adhesives do not etch enamel to the same depth that phosphoric acid does, selective etching of enamel margins has been recommended by some authors prior to the application of self-etch adhesives.[9,10] Manufacturers have also suggested selective enamel etching in the instructions for use of their self-etch adhesives. In noncarious cervical lesions (NCCLs), selective enamel etching did not increase the retention rate in clinical studies when a two-step self-etch adhesive with the ability for chemical bonding was used.[11,12] However, when compared with the self-etch mode, selective enamel etching resulted in an improvement in the enamel marginal integrity at 8 years.[12] The potential drawback of selective enamel etching is that the clinician may inadvertently etch dentin. In fact, bond strengths may decrease when self-etch adhesives are applied on acid-etched dentin when compared with the same adhesive applied in the self-etch mode.[13,14]

Despite unreliable *in vitro* studies and clinical longevity results associated with one-step adhesives when compared with that of adhesives that rely on several steps,[15-17] multimode one-bottle universal adhesives have been developed recently to make the clinical procedure more user-friendly. These new adhesives can be used as self-etch or as etch-and-rinse adhesives. The concept behind these adhesives is novel; hence, only short-term clinical and immediate ultramorphological and bond strength studies have been reported.[18-20]

The aims of this randomized double-blind clinical trial were to study the influence of different application strategies on the clinical behavior of a new universal multimode adhesive (Scotchbond Universal Adhesive, SU, 3M ESPE, St Paul, MN, USA) placed in NCCLs, over the course of 18 months, using two evaluation criteria: World Dental Federation (FDI) and United States Public Health Service (USPHS) criteria. The null hypotheses tested were: 1) bonding to NCCLs using the self-etch strategy, associated or not with selective enamel etching or using the etch-and-rinse strategy, applied on dry or moist dentin, would result in similar retention rates over 18 months of clinical service, and 2) different evaluation criteria (FDI or USPHS criteria) would not result in different outcomes for the same data.

MATERIALS AND METHODS

Study Design

The experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statement.[21] This was a randomized, double-blind clinical trial. The study was carried out in the clinic of the State University of Ponta Grossa (UEPG) School of Dentistry from January 2011 to November 2011. All

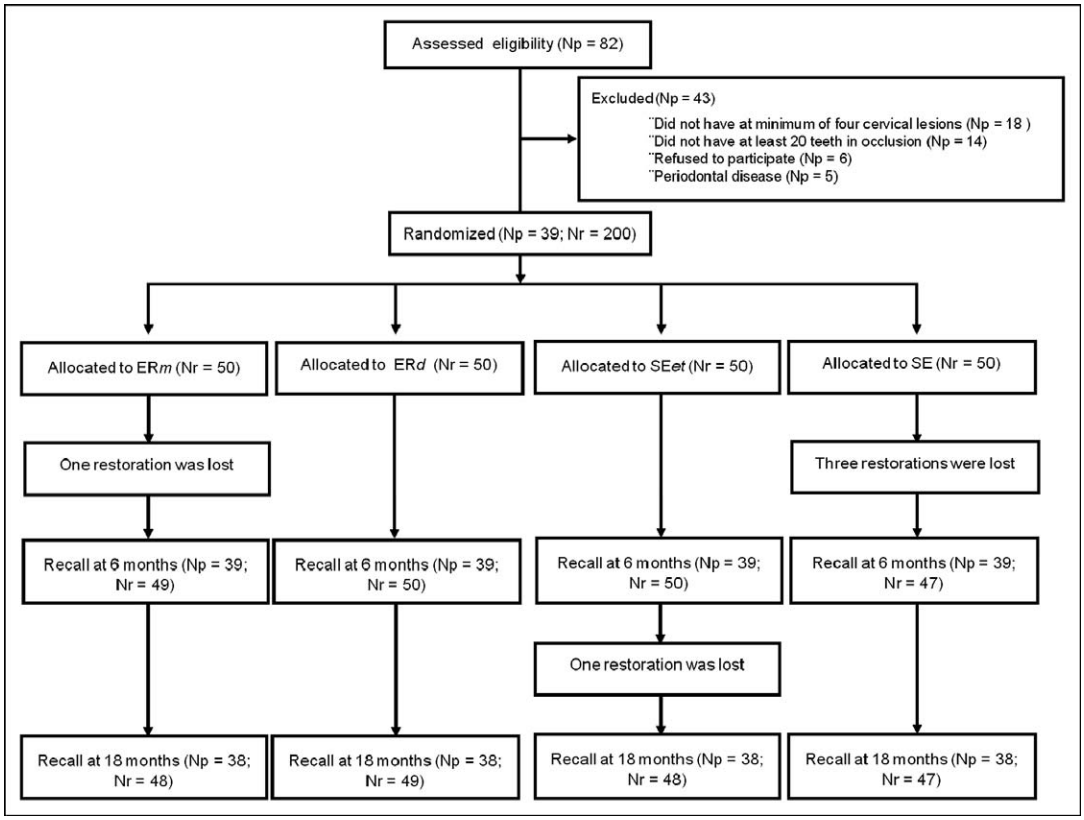

Figure 1. *Flow diagram. ER*d*, total-etch, dry dentin; ER*m*, total-etch, moist dentin; Np, number of patients; Nr, number of restorations; SE, self-etch; S*et*, selective enamel etching.*

participants were informed about the nature and objectives of the study, but they were not aware of what tooth received the specific treatments under evaluation.

Participant Selection

The local Ethics Committee on Investigations Involving Human Subjects reviewed and approved the protocol and consent form for this study (protocol 05909/11). Written informed consent was obtained from all participants prior to starting the treatment. Based on preestablished criteria, 39 volunteers were selected for this study (Figure 1).

Inclusion and Exclusion Criteria

A total of 82 participants were examined by two precalibrated operative dentistry residents to check if they met the inclusion and exclusion criteria (Figure 1). The qualified patients were recruited in the order in which they reported for the screening appointment, thus forming a convenience sample.

The evaluations were performed using a mouth mirror, an explorer, and a periodontal probe. Participants had to be in good general health, be at least 18 years old, have an acceptable oral hygiene level, and present at least 20 teeth under occlusion.

Table 1: *Dentin Sclerosis Scale*[a]

Category	Criteria
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 in category 1 but less than halfway between categories 1 and 4
3	Less sclerosis than in 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

[a] *Adapted from Swift and others.*[23]

Table 2: *Adhesive System: Composition and Application Mode*

Adhesive Systems	Composition/Batch Number	Application Mode[a]			
Scotchbond Universal Adhesive (3M ESPE, St Paul, MN, USA)	1. Scotchbond Universal Etchant: 34% phosphoric acid (UXT-02/Etch-01) 2. Adhesive (UXT-02/Adh-02): methacryloyloxydecyl dihydrogen phosphate, phosphate monomer, dimethacrylate resins, hydroxyethyl methacrylate, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	Etch-and-rinse (ER)	Apply etchant for 15 s Rinse for 10 s Air dry to remove excess water	Keep dentin moist Keep dentin dry, do not overdry	Apply the adhesive for 20 s with vigorous agitation Gently air thin for 5 s Light cure for 10 s
		Selective etching (S*et*)	Apply etchant only on enamel for 15 s Rinse for 10 s Air dry to remove excess water	Keep dentin dry, do not overdry	
		Self-etch (SE)	Do not use etchant	Keep dentin dry, do not overdry	

[a] According to the manufacturer's instructions.

Participants were required to have at least four NCCLs in four different teeth that needed to be restored. These lesions had to be noncarious, non-retentive, deeper than 1 mm, and involve both the enamel and dentin of vital teeth without mobility. The cavo-surface margin could not involve more than 50% of enamel.[22]

All patients were given oral hygiene instructions before the operative treatment was performed. Patients with extremely poor oral hygiene, severe or chronic periodontitis, or heavy bruxism habits were excluded from the study.

Interventions: Restorative Procedure

All of the volunteer participants received dental prophylaxis with a suspension of pumice and water in a rubber cup and signed an informed consent form two weeks before the restorative procedures.

The features of the NCCLs were evaluated prior to the placement of the restorations. The degree of sclerotic dentin was measured according to the criteria described by Swift and others[23] (Table 1). The cavity dimensions in millimeters (height, width, and depth) and the geometry of the cavity (evaluated by profile photograph and labeled at <45°, 45°-90°,

Table 3: *World Dental Federation (FDI) Criteria Used for Clinical Evaluation*[33,34]

	Esthetic Property	Functional Properties	
	1. Staining Margin	2. Fractures and Retention	3. Marginal Adaptation
1. Clinically very good	1.1 No marginal staining	2.1 Restoration retained, no fractures/cracks	3.1 Harmonious outline, no gaps, no discoloration
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
3. Clinically sufficient/ satisfactory (minor shortcomings 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. Clinically unsatisfactory (repair for prophylactic reasons)	1.4 Pronounced marginal staining; major intervention necessary for improvement	2.4 Chipping fractures, which 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
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 *in situ*

90°-135°, >135°) were also recorded. Other features, such as the presence of attrition facets, were also observed and recorded. Preoperative sensitivity was evaluated by applying air for 10 seconds from a dental syringe placed 2 cm from the tooth surface. For the calibration procedure step, the study director placed one restoration of each group in order to identify all steps involved in the application technique. Then, all four operators, who were resident dentists with more than five years of clinical experience in operative dentistry, placed four restorations of each group under the supervision of the study director in a clinical setting. The restoration deficiencies were shown to the operators prior to starting the study. At this point, the operators were considered calibrated to perform the restorative procedures.

The same calibrated operators restored all teeth under the supervision of the study director. All subjects received a minimum of four restorations, one of each experimental group, in different lesions previously selected according to the inclusion criteria.

The randomization process within patients was performed using computer-generated tables by a staff member not involved in the research protocol. Details of the allocated group were recorded on cards contained in sequentially numbered, opaque, sealed envelopes. These were prepared by a staff member not involved in any of the phases of the clinical trial. The allocation assignment was revealed by opening the envelope on the day of the restorative procedure. The operator was not blinded to group assignment when administering interventions; however, participants were blinded to the group assignment.

Before placing the rubber dam, the operators anesthetized the teeth with a 3% mepivacaine solution (Mepisv, Nova DFL, Rio de Janeiro, RJ, Brazil) and cleaned all lesions with pumice and water in a rubber cup, followed by rinsing and drying. Using a shade guide, the proper shade of the composite was determined. Following the guidelines of the American Dental Association (ADA),[24] the operators did not prepare any additional retention or bevel.

The NCCLs received the SU adhesive system applied in different modes: an etch-and-rinse approach, keeping the dentin moist (ER*m*) or dry (ER*d*), and a self-etch approach with (S*et*) or without (SE) selective enamel etching. The compositions, application modes, and batch numbers are described in Table 2.

In the ER*m* group, dentin was kept visibly moist, while in the ER*d* group, dentin was air dried for five seconds but not overdried. In the S*et* group, the lesion was air dried after rinsing the etchant from the enamel. Dentin was kept dry in both the S*et* and SE groups. The adhesive was vigorously agitated on the entire dentin surface in all groups for approximately 20 seconds, according to the manufacturer's recommendations (Table 2). The brush was scrubbed

Table 3: Extended.

	Biological Properties	
	4. Postoperative (Hyper-) Sensitivity	**5. Recurrence of Caries**
1. Clinically very good	4.1 No hypersensitivity	5.1 No secondary or primary caries
2. Clinically good (after correction, very good)	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 shortcomings with no adverse effects but not adjustable without damage to the tooth)	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)	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)	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

Table 4: *Modified United States Public Health Service (USPHS) Criteria According to Bittencourt and Others[35] and Perdigão and Others[30]*

	Marginal Staining	Retention	Fracture
Alfa	No discoloration along the margin	Retained	None
Bravo	Slight and superficial staining (removable, usually localized)	Partially retained	Small chip, but clinically acceptable
Charlie	Deep staining cannot be polished away	Missing	Failure due to bulk restorative fracture

on the dentin surface under manual pressure (equivalent to approximately 45 g or more) followed by gentle air thinning for five seconds and finally light curing (Radii Cal, SDI, Victoria, Australia) for 10 seconds (1000 mW/cm^2).

Filtek Supreme Ultra (3M ESPE, St. Paul, MN, USA) resin composite was used in up to three increments, each one being light cured (Radii Cal, SDI, Victoria, Australia) for 30 seconds. The restorations were finished immediately with fine diamond burs (KG Sorensen, Barueri, SP, Brazil). Polishing was performed with rubber points (Astropol, Ivoclar Vivadent, Liechtenstein) one week after placement of the restorations.

Sample Size Calculation

The sample size calculation was based on the retention rate of the simplified etch-and-rinse Adper Single Bond (3M ESPE), the predecessor of this multimode adhesive from the same manufacturer. The retention rate was reported to be 94% at 18- to 24-month follow-ups.[25-30] Using an α of 0.05, a power of 80%, and a two-sided test, the minimal sample size was 50 restorations in each group in order to detect a difference of 20% among the tested groups.[31]

Clinical Evaluation

Two experienced and calibrated dentists, not involved with the placement of the restorations and therefore blinded to the group assignment, performed the evaluation. For training purposes, the examiners observed 10 photographs that were representative of each score for each criterion. They evaluated 10 to 15 patients each on two consecutive days. These subjects had cervical restorations, and they did not participate in this project. An intraexaminer and interexaminer agreement of at least 85% was necessary before beginning the evaluation.[32]

All parameters during evaluation were recorded using a standardized paper case report form. The evaluation paper was sent to the research staff after each observation, so that the evaluators were blinded to group assignment during follow-up recalls.

The restorations were evaluated by two criteria: the FDI criteria[33,34] and the classical USPHS criteria adapted by Bittencourt and others[35] and Perdigão and others[30] at baseline and after 6 and 18 months of clinical service.

For either of the two criteria, only the clinically relevant measures of performance for adhesives were evaluated. Therefore, wear and color match were not evaluated (Tables 3 and 4). The primary clinical endpoint was restoration retention/fractures, but the following secondary endpoints were also evaluated: marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries. The evaluation of the postoperative sensitivity was performed one week after the restorative procedure by applying air for 10 seconds from a dental syringe placed 2 cm from the tooth surface.

These variables were ranked according to the criteria in the following scores: 1) FDI criteria (clinically very good, clinically good, clinically sufficient/satisfactory, clinically unsatisfactory and clinically poor) and USPHS criteria (*alfa*, *bravo*, and *charlie*). In the case of marginal staining and marginal adaptation, the semiquantitative criteria (SQUACE) proposed by Hickel and others was used.[33,34] Each evaluator outlined the extent of the observed event on a sketch of each restoration using a pen and according to defined criteria (marginal staining and marginal adaptation); after that, each margin was assessed quantitatively as a proportion of the total length of the margin.

Both examiners evaluated all the restorations once and independently. When disagreements occurred during the evaluations, they had to reach a consensus before the participant was dismissed.

The restoration retention rates were calculated according to the ADA guidelines.[24] Cumulative failure percentage = $[(PF + NF)/(PF + RR)] \times 100\%$, where PF is the number of previous failures

Table 4: Extended.

	Marginal Adaptation	Postoperative Sensitivity	Recurrence of Caries
Alfa	Restoration is continuous with existing anatomic form	No postoperative sensitivity directly after the restorative process and during the study period	No evidence of caries contiguous with the margin
Bravo	Detectable V-shaped defect in enamel only; catches explorer going both ways	—	—
Charlie	Detectable V-shaped defect to dentin-enamel junction	Sensitivity present at any time during the study period	Evidence of presence of caries

before the current recall, NF is the number of new failures during the current recall, and RR is the number of currently recalled restorations.

Statistical Analysis

The statistical analyses followed the intention-to-treat protocol according to CONSORT suggestion.[21] This protocol includes all participants in their originally randomized groups, even those who were not able to keep their scheduled recall visits. This approach is more conservative and less open to bias.

Descriptive statistics were used to describe the distributions of the evaluated criteria. Statistical analysis for each individual item was performed, as well as for each overall parameter (FDI criteria). The differences in the ratings of the four groups after 18 months were tested with the Friedman repeated-measures analysis of variance by rank (α=0.05), and differences in the ratings of each group at baseline and after 18 months were evaluated using the McNemar test (α=0.05). Data from SQUACE was categorized into three scores: 1) marginal discrepancies involving less than 10% of the total length of the restoration, 2) between 10% and 30%, and 3) more than 30%, and the groups were compared with Kruskall-Wallis and Mann-Whitney nonparametric tests (α=0.05). Cohen's kappa statistic was used to test interexaminer agreement.

RESULTS

Forty-three of 82 patients were not enrolled in the study because they did not fulfill the inclusion criteria. Thus, 39 subjects (28 patients with four restorations and 11 patients with eight restorations) were selected. All baseline details relative to the research subjects and characteristics of the restored lesions are displayed in Table 5. The overall Cohen's Kappa statistics showed excellent agreement between the examiners at the 6-month (0.94) and 18-month (0.92) follow-up. All research subjects were evaluated at baseline and at six months, and only one patient did not attend the 18-month recall (moved to another city; Figure 1).

Retention

Four restorations were lost at six months (one for ER*m* and three for SE). One restoration was lost between the six- and the 18-month recall (one for S*et*). According to FDI and USPHS criteria, the 18-month retention rates (95% confidence interval) were 98% (90%-100%) for ER*m*, 100% (93%-100%) for ER*d*, 98% (90%-100%) for S*et*, and 94% (84%-98%) for SE. There was no statistical difference between any pair of groups at 18-month recall and for each group when baseline and 18-month times was compared ($p>0.05$; Tables 6 and 7).

Postoperative Sensitivity

Eight restorations had postoperative sensitivity at the 18-month recall using both the FDI and USPHS criteria (three for ER*m*, three for ER*d*, and two for S*et*) with no statistically significant difference when comparing different pairs of groups and for each group when baseline and 18-month times were compared ($p>0.05$; Tables 6 and 7).

Marginal Adaptation

Seventy-nine restorations were considered to have minor discrepancies in marginal adaptation at the 18-month recall using the FDI criteria (19 for ER*m*, 20 for ER*d*, 18 for S*et*, and 22 for SE). No significant difference was detected between any pair of groups at the 18-month recall for either criteria ($p>0.05$). However, a significant difference was detected when baseline and 18-month data were compared within each group ($p<0.05$). Despite these minor discrepancies, only nine restorations were considered to have clinically relevant discrepancies in marginal adaptation (one for ER*m*, five for ER*d,* and three for SE, $p>0.05$; Table 6). When the USPHS criteria were used, nine restorations were scored as *bravo* for marginal adaptation (one for ER*m*, five for ER*d*, and

Table 5: *Distribution of Noncarious Cervical Lesions According to Research Subject (Gender and Age) and Characteristics of Class V Lesions (Shape, Cervicoincisal Size of the Lesion, Degree of Sclerotic Dentin, Presence of Antagonist, Presence of Attrition Facets, Presence of Preoperative Sensitivity, and Tooth and Arch Distribution)*

Characteristics of Research Subjects	Number of Lesions/ Subjects			
Gender distribution				
Male	24			
Female	15			
Age distribution, y				
20-29	05			
30-39	12			
40-49	12			
>49	10			
Characteristics of Class V lesions	ER*m*	ER*d*	S*et*	SE
Shape, ° of angle				
<45				
45-90	14	14	12	12
90-135	34	34	38	38
>135	02	02	00	00
Cervico-incisal height, mm				
<1.5	06	03	05	07
1.5-2.5	29	36	29	30
2.5-4.0	12	10	14	12
>4.0	03	01	02	01
Degree of sclerotic dentin				
1	20	18	17	19
2	12	14	13	10
3	12	12	09	09
4	06	06	11	12
Presence of antagonist				
Yes	50	50	50	50
No	00	00	00	00
Attrition facet				
Yes	24	22	18	26
No	26	28	32	24
Preoperative sensitivity (spontaneous)				
Yes	50	50	50	50
No	00	00	00	00
Preoperative sensitivity (air dry)				
Yes	24	26	30	28
No	26	24	20	22
Tooth distribution				
Anterior				
Incisor	02	02	02	02
Canines	04	08	06	08
Posterior				
Premolar	32	26	34	28
Molar	12	14	08	12
Arch distribution				
Maxillary	25	26	30	28
Mandibular	25	24	20	22

three for SE, $p>0.05$). No significant difference was detected between any pair of groups at the 18-month recall for either criteria and for each group when baseline and 18-month times were compared for both criteria ($p>0.05$).

When SQUACE[33,34] was used, there was a statistical difference among groups at the 18-month evaluation ($p<0.007$; Table 8). SE resulted in a significantly greater number of restorations, with more than 30% of the total length of the interface showing marginal discrepancy (14 restorations) in comparison with the other groups (four for ER*m*, three for ER*d*, and four for S*et*). Also, when baseline vs 18-month results were compared, SE was the only group for which a significantly greater percentage of the interface showed marginal discrepancy at 18 months ($p<0.05$).

For the USPHS modified criteria, nine restorations were classified as *bravo* for marginal adaptation (one for ER*m*, five for ER*d*, and three for SE). No significant difference was found between any pair of groups at the 18-month recall. Likewise, no significant difference was found for each group when baseline and 18-month marginal adaptation data were compared ($p>0.05$; Table 7).

Marginal Staining

Marginal staining was observed in 15 restorations (four for ER*m*, five for ER*d*, two for S*et*, and four for SE) according to FDI criteria. No significant difference was found between groups at 18 months and within each group when baseline and 18-month data were compared ($p>0.05$). For the USPHS-modified criteria, 11 restorations were classified as *bravo* for marginal staining (three for ER*m*, three for ER*d*, two for S*et*, and three for SE), and no significant difference was found between any pair of groups at the 18-month recall. No statistical difference was measured when the baseline and 18-month results were compared within each group ($p>0.05$; Table 7).

Other Parameters

No restoration had clinical problems related to fracture and recurrence of caries at 18 months for

Table 6: *Number of Evaluated Restorations for Each Experimental Group According to the Adhesive (ER*m *[Etch-and-Rinse, Moist Dentin]; ER*d *[Etch-and-Rinse, Dry Dentin]; S*et *[Self-Etch, Selective Enamel Etching]; SE [Self-Etch, No Etching]) Classified According to the World Dental Federation (FDI) Criteria*[33,34]

FDI Criteria	Time	Baseline				6 mo				18 mo			
		ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE
Marginal staining	VG	50	50	50	50	50	50	50	49	45	44	46	45
	GO	—	—	—	—	—	—	—	1	1	2	—	1
	SS	—	—	—	—	—	—	—	—	3	3	2	3
	UN	—	—	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—	—	—
Fractures and retention	VG	50	50	50	50	49	50	50	47	48	49	48	46
	GO	—	—	—	—	—	—	—	—	—	—	—	—
	SS	—	—	—	—	—	—	—	—	—	—	—	—
	UN	—	—	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	1	—	—	3	1	—	1	3
Marginal adaptation	VG	50	50	50	50	34	32	29	27	29	29	30	24
	GO	—	—	—	—	16	18	21	23	18	15	18	19
	SS	—	—	—	—	—	—	—	—	1	5	—	3
	UN	—	—	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—	—	—
Postoperative (hyper-) sensitivity	VG	49	46	47	49	50	50	50	50	45	46	46	46
	GO	—	—	—	—	—	—	—	—	—	—	—	—
	SS	1	4	3	1	—	—	—	—	3	3	2	—
	UN	—	—	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—	—	—
Recurrence of caries	VG	50	50	50	50	50	50	50	50	48	49	48	46
	GO	—	—	—	—	—	—	—	—	—	—	—	—
	SS	—	—	—	—	—	—	—	—	—	—	—	—
	UN	—	—	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations: VG, clinically very good; GO, clinically good; SS, clinically sufficient/satisfactory; UN, clinically unsatisfactory; PO, clinically poor.

either the FDI or the USPHS criteria. When the FDI criteria for "acceptable" vs "not acceptable" restorations were applied, only the five lost restorations were ranked as "not acceptable" (Table 9).

DISCUSSION

New criteria for evaluating dental restorations were published in 2007 and named the "FDI criteria," as a result of the efforts of the FDI to organize them.[33,34] Nonetheless, only a few publications have used the FDI criteria since then.[20,36-38] Most clinical studies reporting clinical evaluation of NCCL restorations still use the USPHS criteria.[23,25-30,39-42] One study,[36] published as an abstract, concluded that the FDI criteria were more sensitive for identifying differences in the restorations than the USPHS criteria. A more recent publication[20] compared the six-month clinical behavior of several adhesion strategies using both FDI and USPHS-modified criteria. The findings suggested that the FDI criteria are more sensitive than the USPHS-modified criteria to small variations in the clinical outcomes when evaluating restorations of NCCLs. This finding was corroborated in the present study, as the marginal discrepancies were more frequently measured in the FDI criteria in relation to USPHS criteria.

Under the USPHS criteria, only nine restorations were scored as *bravo* after 18 months of clinical service, and no significant difference was detected among groups. This percentage is very similar to what was previously reported in clinical trials that used the USPHS-modified criteria after 18 months of evaluation.[23,25,35,43,44] On the other hand, marginal integrity was statistically worse for SE when assessed with the FDI criteria, specifically when SQUACE was used.

SU is considered an ultra-mild self-etch adhesive because its pH is relatively high (3.0; data not

Table 7: *Number of Evaluated Restorations for Each Experimental Group According to the Adhesive (ER*m *[Etch-and-Rinse, Moist Dentin]; ER*d *[Etch-and-Rinse, Dry Dentin]; Set [Self-Etch, Selective Enamel Etching]; SE [Self-Etch, No Etching]) Classified According to the Adapted United States Public Health Service (USPHS) Criteria*[30,35]

USPHS Criteria	Time	Baseline				6 mo				18 mo			
		ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE
Marginal staining	*Alfa*	50	50	50	50	50	50	50	49	45	46	46	43
	Bravo	—	—	—	—	—	—	—	1	3	3	2	3
	Charlie	—	—	—	—	—	—	—	—	—	—	—	—
Retention	*Alfa*	50	50	50	50	49	50	50	47	48	49	48	46
	Charlie	—	—	—	—	1	—	—	3	1	—	1	3
Fracture	*Alfa*	50	50	50	50	50	50	50	50	48	49	48	46
	Bravo	—	—	—	—	—	—	—	—	—	—	—	—
	Charlie	—	—	—	—	—	—	—	—	—	—	—	—
Marginal adaptation	*Alfa*	50	50	50	50	50	50	50	48	47	44	48	43
	Bravo	—	—	—	—	—	—	—	2	1	5	—	3
	Charlie	—	—	—	—	—	—	—	—	—	—	—	—
Postoperative sensitivity	*Alfa*	49	46	47	49	50	50	50	50	45	46	46	46
	Bravo	1	4	3	1	—	—	—	—	—	—	—	—
	Charlie	—	—	—	—	—	—	—	—	3	3	2	—
Recurrence of caries	*Alfa*	50	50	50	50	50	50	50	50	48	49	48	46
	Charlie	—	—	—	—	—	—	—	—	—	—	—	—

shown). This high pH may explain the significant deterioration of marginal adaptation from baseline to 18 months for SE, especially when compared with S*et*. The less pronounced etching pattern of SE when compared with the results of previous studies in which phosphoric acid was applied prior to self-etch application[45-47] may explain these findings. It is worth mentioning that the marginal discrepancies were typically observed in the enamel margins, and this occurrence was not deemed as a clinical failure as it can usually be solved by repolishing the restoration.[48,49]

Despite the use of two clinical evaluation criteria, the most important parameter for the evaluation of NCCL restorations has been retention rate. If the restorations are lost, all of the other criteria cannot be evaluated. In general, the clinical behavior of SU at 18 months in this study, regardless of the bonding strategy, was very good and comparable to that of the three-step etch-and-rinse adhesive Adper Scotchbond Multi-Purpose (3M ESPE) and that of the two-step etch-and rinse adhesive Adper Single Bond Plus (3M ESPE).[30]

SU differs from Adper Single Bond Plus adhesive primarily by the incorporation of the 10-MDP monomer in the former, to provide acidity for its self-etching capability. Chemical bonding between 10-MDP and enamel/dentin may have resulted in stable interfaces even without micromechanical retention from etching in the SE group.[50,51] The success of 10-MDP has been reported in the literature. Its intrinsic chemical bonding, combined with the good mechanical properties and high conversion rate of a filled hydrophobic resin,[52,53] resulted in very good clinical behavior of Clearfil SE Bond (CSE, Kuraray, Osaka, Japan) at eight years.[12]

The clinical behavior of SU in our study was also similar to that of Adper Easy Bond (AEB, 3M ESPE), a one-step self-etch adhesive, in a recent 18-month clinical study,[43] regardless of the application of a hydrophobic resin layer over AEB. This similarity in clinical behavior between these two adhesives from the same manufacturer may have to do with the comparable concentration of the polyalkenoic acid copolymer in both materials (1%-5%).[54,55] This copolymer may also provide chemical bonding derived from its spontaneous bonding to hydroxyapatite.[56] The polyalkenoic acid copolymer was first used in the composition of Vitrebond (3M ESPE) and therefore is also known as Vitrebond copolymer, or VCP. For self-etch adhesives, chemical bonding between polycarboxylic monomers (such as VCP) and hydroxyapatite plays a crucial role in their bonding mechanism.[57,58] More than 50% of the carboxyl groups in the polyalkenoic acid copolymer are capable of bonding to hydroxyapatite.[57] Carboxylic groups replace phosphate ions on the substrate and make ionic bonds with calcium.[57]

Table 8: *Number of Evaluated Restorations for Each Experimental Group According to the Adhesive Classified for Semiquantitative Score (SQUACE)*[33,34]

FDI Criteria	Statistical Analysis[a]	ER*m*	ER*d*	S*et*	SE
SQUACE	Less than 10%	0	0	1	0
	Between 10% and 30%	12	13	14	14
	Between 31% and 50%	4	3	4	14
		A	A	A	B

*Abbreviations: ER*d*, etch-and-rinse, dry dentin; ER*m*, etch-and-rinse, moist dentin; SE, self-etch, no etching; S*et*, self-etch, selective enamel etching.*
[a] *Different letters indicate significant differences between groups (Kruskall-Wallis, p=0.007).*

Taking into account that SU contains two molecules with a potential for chemical bonding (10-MDP and VCP), the clinical behavior of SE and S*et* in our study may have been a result of: 1) the formation of a submicron micromechanical interlocking at the dentin surface by SU,[51] 2) the chemical bonding of both the 10-MDP monomer and VCP to hydroxyapatite, or 3) the protective effect of the Ca-MDP salt, which is a very hydrolytically stable salt.[59] However, it is worth mentioning that, although CSE resulted in nano layering within the hybrid layer and into the adhesive layer, SU resulted in nano layering only at the tubule orifices, where the adhesive infiltrated the residual smear layer.[51] The difference between these two adhesives may rely not only on the higher concentration of 10-MDP in CSE, but also on the presence of hydroxyethyl methacrylate (HEMA) in SU, which may prevent interfacial self-assembled nanolayering.[60] Therefore, further data from subsequent clinical recalls should be compared with those of CSE[12] to validate this compositional difference between SU and CSE. In fact, nano layering of two 10-MDP molecules may result in an adhesive interface more resistant to degradation.[60]

At 18 months, there were no differences in postoperative sensitivity between any pair of groups in the present study. Nevertheless, it is noteworthy that SE did not result in any restoration with postoperative sensitivity. Other clinical studies in NCCLs have shown no difference in postoperative sensitivity between self-etch and etch-and-rinse adhesives.[30,61,62] Self-etch adhesives use part of the smear layer as the bonding substrate; therefore, the monomer-impregnated smear plugs serve as a barrier to prevent the fluid shift inside the tubules. It has been reported that self-etch adhesives result in a simultaneous demineralization and infiltration of the dentin substrate.[12] Nevertheless, incomplete infiltration of dentin by mild self-etch adhesives has been reported,[63] because these adhesives have a reduced etching potential toward the base of hybrid layers.[63] On the other hand, etching dentin with phosphoric acid has been associated with reduced bond strengths with self-etch adhesives.[13,14] However, more recent studies have demonstrated that the effects of intentional dentin etching with phosphoric acid prior to the application of self-etch adhesives are material dependent.[64,65]

One-step self-etch adhesives are highly hydrophilic; therefore, they attract water and may increase the potential for degradation,[66,67] as water sorption of adhesive resins is proportional to their hydrophilic characteristics.[68] The self-etching ability of self-etching primers is achieved by incorporating sufficient water in the solution for adequate ionization of the acidic monomers without lowering the monomer concentration to a threshold that would compromise the bonding efficacy. Water is an important ingredient because it ionizes the acidic groups, allowing the formation of hydronium ions (H_3O^+), which etch

Table 9: *Restorations Acceptable or Not Acceptable According to the Federation Dental International (FDI) Criteria After 6 Months*[33,34]

Properties	Esthetic				Functional								Biological							
	Staining Margin				Fractures and Retention				Marginal Adaptation				Postoperative (Hyper-) Sensitivity				Recurrence of Caries			
	ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE	ER*m*	ER*d*	S*et*	SE
Acceptable	46	48	44	43	45	48	43	40	46	48	44	43	46	48	44	43	46	48	44	43
Not acceptable	00	00	00	00	01	00	01	03	00	00	00	00	00	00	00	00	00	00	00	00
Reasons	Total loss of restoration																			

*Abbreviations: ER*d*, etch-and-rinse, dry dentin; ER*m*, etch-and-rinse, moist dentin; SE, self-etch, no etching; S*et*, self-etch, selective enamel etching.*

hydroxyapatite.[69] Water also facilitates solubilization of the reaction products resulting from the etching process. Increasing the water concentration dilutes the concentration of the acidic monomer and may decrease its bonding effectiveness. The presence of such hydrophilic moieties may induce increased water sorption and water uptake,[70] in turn jeopardizing the stability of the polymer network with time.

With this in mind, further studies should include the use of SU as a two-step self-etch adhesive by adding a hydrophobic resin layer as a second step in the bonding sequence. The laboratory and clinical success of mild two-step self-etch adhesives might also be a result of the presence of a hydrophobic bonding layer.[44,71] Nonetheless, adding a hydrophobic layer to one-step self-etch adhesives may not result in improved clinical performance for all simplified adhesives. A recent 18-month clinical trial reported that the clinical behavior of AEB did not change with the application of an extra layer of a bonding resin.[43] Therefore, the effect of the hydrophobic layer may depend on the specific composition of the one-step self-etch adhesive.

In addition to further recall evaluations already planned for this same project, bond strengths of aged dentin-resin interfaces with the same experimental groups are needed, as they may shed some light on the clinical behavior of SU over five years. While laboratory studies cannot always predict the clinical durability of bonded restorations, the dentin bond strengths of aged specimens seem to correlate with five-year clinical data.[17,72]

This clinical study has limitations, as 18 months is still a short period for evaluating the long-term clinical behavior of any dental adhesive. Nevertheless, SU belongs to a novel generation of simplified adhesives that are indicated for use under different application strategies, although they lack clinical data. Another limitation of the present study is that more than four restorations were placed in several patients, which may have caused a clustering effect. Despite being a common situation in the dental literature,[35,73] the influence of clustering on the data was not computed.

SU fulfilled the ADA guidelines[23] on the basis of the 18-month recall data, as this clinical study demonstrated no greater incidence of clinical failures than 10%, regardless of the bonding strategy used. We failed to reject the first null hypothesis, as there were no statistical differences in the clinical retention rates at 18 months for the different bonding strategies tested in this study. We have to partially reject the second null hypothesis, as significant differences were measured for marginal integrity when using the FDI criteria.

CONCLUSIONS

Within the limitations of this study, the 18-month clinical behavior of Scotchbond Universal Adhesive (3M ESPE) does not depend on the bonding strategy used. The new multimode adhesive fulfilled the ADA criteria for full approval when using all of the bonding strategies suggested by the manufacturer. The FDI evaluation criteria are more sensitive to small variations in the clinical outcomes than the USPHS criteria are, when evaluating restorations of NCCLs.

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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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Administration of Ascorbic Acid to Prevent Bleaching-induced Tooth Sensitivity: A Randomized Triple-blind Clinical Trial

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Clinical Relevance

The use of ascorbic acid during in-office bleaching does not reduce the incidence of bleaching-induced tooth sensitivity.

SUMMARY

This study evaluated the effect of ascorbic acid, 500 mg every eight hours, on bleaching-induced tooth sensitivity. A triple-blind, parallel design, and placebo-controlled randomized clinical trial was conducted on 39 adults. The pills (placebo or ascorbic acid) were administered three times per day for 48 hours; the first dose was given one hour prior to each bleaching session. Two bleaching sessions with 35% hydrogen peroxide gel were performed with a one-week interval. Tooth sensitivity was recorded up to 48 hours after bleaching. The color evaluation was performed before and 30 days after bleaching. The absolute risk and intensity of tooth sensitivity were evaluated by Fisher exact and Mann-Whitney U-tests, respectively. Color changes were evaluated by unpaired *t*-test (α=0.05). There were no significant differences in the absolute risk and intensity of tooth sensitivity and color change between the groups. Both groups showed a similar risk of tooth sensitivity ($p>0.05$). The perioperative use of an antioxidant, such as ascorbic acid (500 mg, three times daily) perorally, was not able to prevent bleaching-induced tooth sensitivity or reduce its intensity.

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INTRODUCTION

The desire for whiter teeth has made tooth bleaching one of the most sought-after cosmetic procedures in

dentistry.[1] Various agents can be used to whiten teeth, such as hydrogen peroxide (HP), carbamide peroxide, and sodium perborate. These materials can penetrate the enamel and dentin structures, releasing reactive oxygen radicals that oxidize chromogens.[2] Available bleaching modalities include dentist-supervised in-office bleaching, dentist-prescribed home-applied bleaching, and over-the-counter consumer-available systems.[3]

The in-office procedure using 35% HP has a long history of tooth sensitivity (TS) and gingival irritation.[3-6] Prevalence levels of TS have been reported to vary from 55% to 90%.[4-8] This sensitivity seems to result from the easy passage of the HP through the enamel and dentin to the pulp,[9] causing pulp damage and inflammation.[10]

This cell damage is probably the result of oxidative stress produced by HP and its by-products on cells. HP is a potential source for hydroxyl radicals, one of the most dangerous radicals. It was reported that HP and its by-products, superoxide anions and hydroxyl radicals, are injurious to cells via oxidative stress,[11] and they were able to cause cytotoxicity, apoptosis, and genotoxicity in mouse P388 cells.[12] Along with cell damage, cell-derived factors, such as ATP[13] and prostaglandins, are released, exciting and sensitizing pulp nociceptors,[14] leading to the transmission of the pain stimuli.

In an attempt to reduce the damage produced by HP on the pulp, some authors[12,15,16] have investigated the role of antioxidants. It was reported that the use of a flavonoid, which is a naturally occurring antioxidant (naringin), on dentin before HP application reduced the H_2O_2-induced cytotoxicity, apoptosis, and genotoxicity[12] under *in vitro* conditions. This antioxidant was also shown to suppress the DNA damage induced by HP. Other authors reported that the application of 10% sodium ascorbate on dentin discs, before the application of a carbamide peroxide gel, reduced the cytotoxic effects of these products on cells,[15] and these effects were shown to be directly related to the HP concentration and the period the antioxidant was left on the tooth surface.[16]

The results of the aforementioned studies indicate that the presence of a natural product with antioxidant and anti-apoptotic properties on pulp could reduce the damage produced by the in-office bleaching products, and this could be clinically translated to a reduction of the risk of TS and its intensity. However, the only available method to deliver an antioxidant to the pulp tissue is through either a peroral route or intravenously. Therefore, this study attempted to investigate if the perioperative use of an antioxidant perorally during in-office bleaching could reduce the oxidative stress produced by HP on pulp cells, and thus, reduce the risk and intensity of bleaching-induced TS. Three null hypotheses were tested: 1) the perioperative use of ascorbic acid, starting one hour before the in-office bleaching session, will not reduce the absolute risk of TS; 2) the use of this antioxidant drug will not reduce the intensity of TS; and 3) the use of this antioxidant will not affect the degree of tooth whitening.

MATERIALS AND METHODS

This clinical investigation was approved (protocol 17836/2010) by the scientific review committee and the committee for the protection of human subjects of the State University of Ponta Grossa (Ponta Grossa, PR, Brazil). The experimental design followed the Consolidated Standards of Reporting Trials statement.[17] Thirty-nine volunteers from the city of Guarapuava, PR, Brazil were selected for this study in the clinic of the Brazilian Association of Dentistry in Guarapuava from May 2011 to June 2012. Two weeks before the bleaching procedures, all of the volunteers received a dental prophylaxis with pumice and water in a rubber cup and signed an informed consent form.

Inclusion and Exclusion Criteria

Participants included in this randomized, triple-blind, placebo-controlled with a parallel-group clinical trial were at least 18 years old and had good general and oral health. Participants were recruited by means of radio and television advertisement. The participants were required to have at least six maxillary and mandibular anterior teeth that were caries-free and without restorations on the labial surfaces. Selected participants had central incisors that were shade C2 or darker, as judged by comparison with a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Germany).

Participants who had previously undergone tooth-whitening procedures or had preexisting anterior restorations or internal tooth discoloration (tetracycline stains, fluorosis, pulpless teeth) were not included in the study. Pregnant and lactating women and participants taking any medicine were not included in the study. Additionally, participants with bruxism habits or any pathology that could cause TS (eg, recession, dentin exposure) were excluded. This was done to minimize confounding experimental variables or side effects from bleaching. Participants who reported a history or presented

health problems in the stomach, heart, kidney, or liver, or participants using any continuous drug with anti-inflammatory and antioxidant action were also excluded from the study.

Study Intervention

Participants were randomly divided into the placebo (n=19 participants) and ascorbic acid groups (n=20 participants). The randomization process was performed using computer-generated tables by a third person (statistician), not involved in the research protocol. Details of the allocated groups were recorded on cards contained in sequentially numbered, opaque, sealed envelopes. Once the participant was eligible for the procedure and completed all baseline assessments, the allocation assignment was revealed when the research assistant opened this envelope. Neither the participant nor the operator (E.A.P.) knew the group allocation, being both blinded to the protocol.

The participants from the placebo group received a placebo pill (Talco pharma S M-200 Henrifarma, São Paulo, SP, Brazil), and participants from the experimental group received a 500 mg dose of ascorbic acid (pill, vitamin C, Citroplex, Lab Neo Química, Anápolis, GO, Brazil). All of the participants were watched to ensure that they took the drugs or placebo one hour prior to treatment. The other doses of placebo or ascorbic acid were administered every eight hours after the first dose over a period of 48 hours. Participants were reminded by a research assistant via telephone to take their doses of ascorbic acid/placebo. This procedure was done to increase adherence to the protocol.

The medicine was administered for 48 hours because, although bleaching-induced tooth sensitivity complaints usually cease within the first 24 hours, some patients have reported pain up to 48 hours after treatment.[18] We have selected the minimal dosage of ascorbic acid available on the Brazilian market. The medicine was administered every eight hours because the concentration of ascorbic acid is almost minimal eight hours after ingestion.[19]

The gingival tissue of the teeth to be bleached was isolated from the bleaching agent using a light-cured resin dam (Top Dam, FGM, Joinville, SC, Brazil). The 35% HP gel (Whiteness HP Maxx, FGM) was used in three 15-minute applications for both groups following the manufacturer's directions. The in-office bleaching agent was refreshed every 15 minutes during the 45-minute application period. Two bleaching sessions, with a one-week interval, were performed on each patient (E.A.P.). All participants were instructed to brush their teeth at least three times a day using a fluoridated toothpaste (Sorriso Fresh, Colgate-Palmolive, São Paulo, SP, Brazil) provided by the investigators.

Shade Evaluation

Shade evaluation was recorded before and 30 days after the bleaching treatment using two methods: subjective evaluation using a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik) and an objective evaluation using the Easyshade spectrophotometer (Vident, Brea, CA, USA).

For the subjective examination, the shade guide's 16 tabs were arranged from highest (B1) to lowest (C4) value, making the minimum qualifying shade C2 as number 7 (seventh tab on the value-ordered arrangement). Although this scale is not linear in the truest sense, we treated the changes as representing a continuous and approximately linear ranking for the purpose of analysis. The measurement area for shade matching was the middle third of the facial surface of the anterior central incisor. This measurement was done at baseline and 30 days after bleaching, allowing for the calculation of means and standard deviations of delta shade guide units (ΔSGU) of each group.

For calibration purposes, five participants whom we did not include in the sample participated in the training phase of this study. The two examiners (A.R. and A.D.L.), blinded to the allocation assignment, scheduled these participants for bleaching and evaluated their teeth against the shade guide at baseline and 30 days after the procedure. The two examiners were required to have an agreement of at least 85% (kappa statistic) before beginning the study evaluation. During the study, if disagreements arose, the examiners reached a consensus before dismissing the patient.

For the objective evaluation, a preliminary impression of the maxillary arch was made using dense silicone Adsil (Vigodent, Rio de Janeiro, RJ, Brazil). The impression was extended to the upper canine and served as a standard shade measurement guide for the spectrophotometer. A window was created on the labial surface of the molded silicone guide for the central incisor to be evaluated. The window was made using a metallic device with well-formed borders, 3 mm in radius.[5] The measurement was done on all 39 participants using the Vita Easyshade spectrophotometer (Easyshade, Vident) before and

30 days after the bleaching therapy by only one operator (E.A.P.). The shade was determined using the parameters of the Easyshade device where it indicated the following values: L*, (a*), and (b*), in which L* represents the value from 0 (black) to 100 (white) and a* and b* represent the shade, where a* is the measurement along the red-green axis and b* is the measurement along the yellow-blue axis. The shade comparison before and after treatment was given by the differences between the two shades (ΔE), which is calculated using the formula[20-22]: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Tooth Sensitivity Evaluation

The patients recorded their perception of TS during the first and second bleaching sessions using two pain scales. A five-point verbal rating scale [0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe][5,6,8] and a visual analog scale[23-26] using a 10-cm horizontal line with words "no pain" at one end and "worst pain" at the opposite end were employed in this study. We asked subjects to record whether they experienced TS during the treatment and up to 48 hours after bleaching.

As two bleaching sessions were performed, the worst scores/numerical values obtained in both bleaching sessions were considered for statistical purposes. The values were arranged into two categories: absolute risk of TS, which was the presence of TS at any assessment point, and intensity of TS at each assessment point. These values were computed only for the maxillary arch.

Statistical Analysis

For sample size calculation, the primary outcome was the absolute risk of TS. The absolute risk of TS was reported to be approximately 90%[8,27-29] for the bleaching product Whiteness HP Maxx (FGM). In order to be able to detect an absolute difference of 40% between the placebo and the experimental group, a minimum of 17 participants were required with a power of 80% and alpha of 5%.

The data analysis followed the intention-to-treat protocol and involved all participants who were randomly assigned.[17] The statistician was blinded to the study groups. The primary outcome absolute risk of TS was compared by using the Fisher exact test (alpha=5%). The relative risk, as well as the confidence interval for the effect size, was calculated.

The data sets of TS intensity were plotted on histograms and inspected for normal distributions. As the data did not appear to be normally distributed, nonparametric statistical tests were used. For each pain scale, a comparison of the two groups at the three different assessment points was performed using the Mann-Whitney U-test. Comparisons between times within each group were performed using the Friedman tests. In all statistical tests, the significance level was 5%.

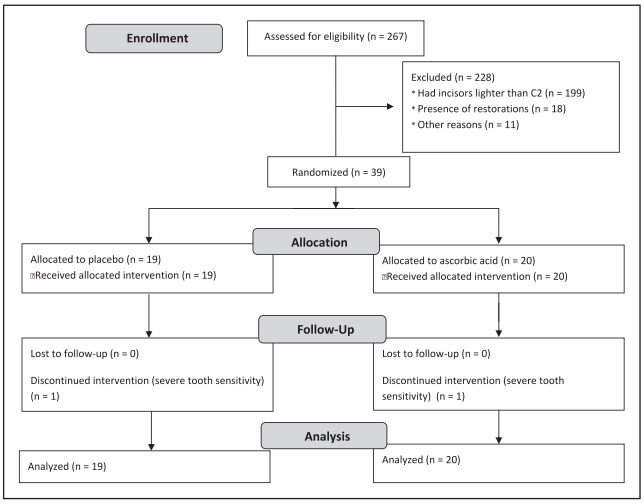

Figure 1. *Flow diagram of the clinical trial, including detailed information on the excluded participants.*

Shade change, another secondary endpoint, was used to assess the efficacy of the bleaching treatment associated with perioperative use of ascorbic acid. The data from ΔSGU and ΔE values of both groups were compared by the Student *t*-test. In all statistical tests, the significance level was set at alpha of 5%.

RESULTS

A total of 267 participants were examined to select 39 participants for the study (Figure 1). The mean ages (years) of the participants in this study were similar between the groups (placebo: 25.3 ± 6.7 years and ascorbic acid: 28.3 ± 9.7 years, *t*-test, $p=0.832$). The baseline colors (SGU) were also similar between the groups (placebo: 9.5 ± 1.9 and ascorbic acid: 9.9 ± 1.8; *t*-test, $p=0.552$). Of the participants from the placebo and ascorbic acid groups, 53% and 35% were male, respectively. Figure 1 depicts the participant flow diagram in the different phases of the study design.

Tooth Sensitivity

The data from 39 participants were used in this study, following the intention-to-treat analysis. One patient from the ascorbic acid group received an

Table 1: *Comparison of the Number of Patients Who Experienced Tooth Sensitivity (TS) at Least Once During the Bleaching Regimen in Both Groups Along With Absolute and Relative Risks**

Treatment	Number of Participants With TS		Absolute Risk (95% CI)	Relative Risk (95% CI)
	Yes	No		
Placebo	16	3	84.2 (62.4-94.5)	1.05 (0.78-1.41)
Ascorbic acid	16	4	80 (58.4-91.9)	

* Fisher test (p=1.0).

analgesic after the first bleaching session, and one patient from the placebo group received an analgesic after the second bleaching session due to severe TS.

With regard to the absolute risk of TS, no significant difference was observed between groups (Table 1; $p=1.00$). The relative risk, along with the 95% confidence interval, is also evidence that the use of the experimental drug had no effect on the TS reduction.

Most of the TS complaints occurred within the first 24 hours, and only two participants experienced pain after 24 hours. With regard to TS intensity (Table 2), the groups did not differ statistically under the two pain scales used in this study ($p>0.05$).

Significant whitening was observed in both study groups under the subjective and objective evaluation methods ($p<0.001$). A whitening of approximately 5.7 and 5.6 SGU were detected for placebo and ascorbic acid groups, respectively (Table 3). A variation of 8.0 and 7.0 in the ΔE was observed for the placebo and ascorbic acid groups, respectively (Table 3). The results of the subjective (visual shade guide) and the objective evaluation (spectrophotometer) matched the hypothesis of equality between the groups after bleaching ($p \asymp 0.6$ for both methods).

DISCUSSION

HP is a reactive oxygen species (ROS) frequently found within the cells as the result of a series of intracellular reactions that occur specifically in the mitochondria.[30] Whether produced endogenously as a consequence of normal cell functions or derived from external sources, ROS pose a constant threat to living cells because they can cause severe damage to DNA, protein, and lipids. Cells contain a number of antioxidant defenses to minimize fluctuations in ROS, but when ROS generation and/or exposure exceeds the antioxidant capacity of cells, a condition termed oxidative stress occurs.[31] This may be the cause of pulp damage produced by in-office bleaching.[10]

Although HP, by itself, is relatively nonreactive toward DNA, most of the HP-mediated damage is due to the production of the hydroxyl radical, a by-product of the HP degradation. The hydroxyl radical is an extremely reactive oxidant; it can react rapidly with DNA and can cause over 100 different types of DNA modification.[30] Therefore, the increase in the exogenous levels of these highly reactive free radicals in contact with cells, as it occurs during in-office bleaching with HP, may result in cell death and reduction of cell proliferation.[32-35]

As mentioned in the Introduction, several studies have proposed the use of antioxidant agents for treatment and/or prevention of the oxidative stress caused by HP from bleaching,[12,15,16] and promising findings have been reported. Among antioxidants available for oral administration, ascorbic acid, also known as vitamin C, is the most popular, and gram doses are promoted for preventing and treating the common cold, managing stress, and enhancing well-

Table 2: *Comparison of Tooth Sensitivity Intensity Experienced by Patients From the Treatment Groups at Different Assessment Points Using Two Pain Scales**

Time Assessment	0-4†		0-10†	
	Placebo	Ascorbic Acid	Placebo	Ascorbic Acid
Up to 1 hour	2 (0/2) aA	1 (0/2.75) aA	2.2 (0/3.2) aA	1.6 (0/3.3) aA
1 to 24 hours	2 (0/2) aA	1 (0/3) aA	1.6 (0/4.7) aA	2.2 (0/3.2) aA
24 to 48 hours	0 (0/0) aB	0 (0/0) aB	0 (0/0) aB	0 (0/0) aB

** Comparisons are valid only within the same pain scale. At each period, the two treatments were compared with the Mann-Whitney U-test and differences are represented by different lowercase letters. For each treatment, the three periods were compared with the Friedman test ($\alpha=0.05$,), and differences are represented by different uppercase letters. † Medians (first/third quartile) values.*

Table 3: *Means and Standard Deviations of the Change in Shade Guide Units (Vita Classical Shade Guide, [ΔSGU]) and ΔE (Spectrophotometer) Between Baseline and 30 Days After Bleaching for the Two Treatment Groups**

		Placebo	Ascorbic Acid	*p*-Value
Subjective evaluation	ΔSGU	5.7 ± 1.8 A	5.6 ± 2.9 A	0.86
Objective evaluation	ΔE	8.0 ± 3.0 A	7.0 ± 3.6 A	0.28

** Comparisons are only valid within rows. Means indicated by the same uppercase letters indicate statistically similar means (Student t-test, α=0.05).*

being. Ascorbic acid is an electron donor, and because of this, is a potent water-soluble antioxidant in humans.[36] In addition, ascorbic acid has been studied for the treatment of several well-known diseases such as hypertension[37] and cancer,[38] due to its ability to minimize the harmful effect of free radicals, reducing the oxidative stress on cells.

However, contrary to the current authors' expectations, the perioperative use of ascorbic acid did not reduce the bleaching-induced TS, which led us not to reject the first and second null hypotheses. Unfortunately, results from *in vitro* studies cannot necessarily be extrapolated to the clinical situation. It is probable that the amount of antioxidant delivered to cultured cells in the *in vitro* studies[12,15,16] was much higher than the level of antioxidant reached in the extracellular fluid after oral administration of 500 mg of ascorbic acid. By using the peroral route of administration, several factors such as the presence of the immune system, lymphatic drainage, urinary excretion, and morphologic characteristics of the dentin substrate may modulate the amount of ascorbic acid that reaches the plasma and extracellular fluid[19] around pulp cells.

Clinical pharmacokinetic studies have shown that ascorbic acid concentrations in plasma and tissues were tightly controlled under oral administration.[39] At doses lower than 100 mg/day, there is a steep sigmoidal relationship between dose and concentrations. At doses higher than 100 mg/day, plasma concentrations reach a plateau between 70 and 80 μmol/L. At doses greater than 400 mg/day, further increases in plasma concentrations were minimal.[39] Thus, the administration of higher dosages of ascorbic acid than the one given in this study would not be expected to provide additional benefits.

On the other hand, when ascorbic acid is administered intravenously, the limiting absorptive mechanism is bypassed, and high plasma levels are attained.[19] For instance, following the administration of 1.25 g intravenously, a peak plasma level of 1000 μmol/L is reached, even though 100 μmol/L is not exceeded by oral dosing.[40] Therefore, one could speculate that the intravenous administration of ascorbic acid could be an option to increase the concentration level of ascorbic acid within and around pulp cells, although intravenous administration is not suitable for routine use in bleaching procedures.

Another option would be the topical application of ascorbic acid on the enamel surface, as a way to allow fast delivery of the antioxidant to the pulp tissue; however, it is probable that the ascorbic acid would not penetrate the enamel substrate, by diffusion alone, due to its organic composition and high molecular weight (176.09 g/mol). This may be a possibility by using dielectrophoresis to drive drugs directly into site-specific intraoral targets. This technology could transport drugs "directly" into teeth using an alternating current electric field. When this technology becomes clinically feasible, it may reduce the oral systemic route of many drugs, overcoming the disadvantages of peroral route administration, and allowing fast delivery of drugs to the pulp tissue.[41]

With regard to the bleaching outcome, the results of this study indicated that both groups demonstrated equivalent and significant tooth shade enhancement when compared with the baseline (Table 3), and thus the third null hypothesis was not rejected. The comparison of shade change after in-office bleaching with existing literature is difficult, due to the different methods of measurement (shade guides and spectrophotometers) and different units of measurement (eg, CIELab system, shade guide units) employed. However, studies that used 35% HP and reported their results in shade guide units usually observed an overall shade change of 5 to 8 shade guide units after two bleaching sessions,[6,42-45] which is in agreement with the results of the present investigation.

This study was designed to find a high effect size, ie, a difference in 40% in the TS among participants from the experimental and placebo groups. Thus, we can conclude that an effect as large as this was not observed, but we cannot rule out the fact that smaller effect sizes do exist. Conducting the same

experimental design using higher sample sizes should be encouraged to rule out this hypothesis. Additionally, the sample selected is mainly composed of young participants, which also limits the ability to generalize for older adults.

CONCLUSIONS

Within the limitations of the current study, we conclude that the use of ascorbic acid, 500 mg three times daily, does not reduce the absolute risk and intensity of TS. However, this study was designed to detect a high effect size, and thus we cannot entirely rule out the benefits of ascorbic acid on TS.

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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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Randomized Clinical Trial on the Efficacy and Safety of Four Professional At-home Tooth Whitening Gels

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Clinical Relevance

Based on the results obtained in this study, in which no statistical differences in the degree of whitening with the different gels were found, we would recommend the use of gels at a lower concentration.

SUMMARY

Objective: This randomized clinical trial evaluated the efficacy and safety of four gels of differing concentrations used for at-home vital bleaching.

Materials and Methods: Ninety-six volunteers participated in the study and were divided into four groups of 24 individuals. A gel of differing concentration was used for each group: 10% and 15% carbamide peroxide and 7.5% and 9.5% hydrogen peroxide. The patients used the whitening agent in a tray without reservoirs for one hour per day for two weeks. The measurement of the change in tooth color was made by two observers in the maxillary right central incisor and with a colorimeter in both upper central incisors and canines, using the CIE L*a*b* and CIE L*C*h* values. Sensitivity was evaluated by the participants on a scale with values as follows: 0 = absent, 1 = minor, 2 = moderate, 3 = considerable, 4 = severe.

Results: At the baseline, the observers noted darker colors than the colorimeter ($p<0.01$), and there were differences between incisors and canines in all the CIE L*a*b* and CIE L*C*h* values ($p<0.001$). In all of the groups and for all of the CIE L*a*b* and CIE L*C*h* parameters, there were color changes in the assessments made in the four maxillary teeth after treatment ($p<0.001$). There were no differences in ΔL* and ΔE* between the groups. The number of patients who experienced sensitivity and the intensity of the sensitivity were not significant.

Conclusions: There were no differences in the degree of whitening among the different products. With all of the products there was an increase in L*, a decrease in chromatic inten-

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sity (C*), and an increase in the value (tone) or hue (h*).

INTRODUCTION

Ever since Haywood and Heymann[1] described night-guard vital bleaching in 1989, there have been various concentrations of hydrogen peroxide (HP) and carbamide peroxide (CP) used for at-home vital bleaching.[2,3] The American Dental Association only considers 10% CP to be a safe whitener.[4] The Scientific Committee on Consumer Products of the European Commission states that those whitening products with a HP concentration higher than 6% cannot be considered safe for the consumer.[5]

The principal compound responsible for dental whitening is HP (H_2O_2).[6] A 10% CP gel is composed of approximately 3.5% H_2O_2 and 6.5% urea.[7] Therefore, a 3.5% HP concentration should have a whitening effect similar to a 10% CP.[8] A 25% CP gel is composed of 8.7% H_2O_2 and thus would be the equivalent of a 7.5% HP gel.[9]

There are different protocols in the daily application time for gels applied with a tray: 30 minutes, one hour, two hours, and up to eight hours overnight.[10] Dental sensitivity and gingival irritation are well-known side effects.[11-13] Numerous clinical studies demonstrate the effectiveness of at-home vital bleaching.[14] The evaluation of the whitening effectiveness is made through observation and color guides, in particular the Vitapan Classical guide (Vita Zahnfabrik, Bad Säckingen, Germany)[15-18]; the colorimeter[10,19-21]; image digitalization; and software evaluation.[22-25]

The Commission Internationale d'Eclairage developed the CIE L*a*b* system.[26] It is a tridimensional grid in which all colors visible to the human eye are located. Colorimeters or digital spectrophotometers measure color based on the following parameters: the lightness of the color, L* (0 being black and 100 being white); a*, in which negative values indicate green and positive values indicate red; and b*, in which negative values indicate blue and positive values yellow. The difference between colors is given by the formula $\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$, which does not express the direction in which the color deviation is oriented. Based on the CIE L*a*b*, CIE L*C*h* can be determined, whereby $C^* = \sqrt{a^{*2} + b^{*2}}$ and represents chroma or saturation. This ranges from 0, which represents no saturation (ie, a neutral gray, black, or white), to 100 or more for very high chroma (saturation) or "color purity." The hue, $h^* = b/\sqrt{a^{*2} + b^{*2}}$, indicates the tone and is expressed in degrees and thus the position of the color within a sphere. On converting the L*a*b* coordinates to L*C*h*, positive numbers are produced, and since each value represents a characteristic of the color (lightness, saturation, and tone), it is easier to interpret the data. The differences between two values are determined as $\Delta L^* = L^*_1 - L^*_0$, $\Delta a^* = a^*_1 - a^*_0$, $\Delta b^* = b^*_1 - b^*_0$, $\Delta C_{ab}^* = C^*_{ab,1} - C^*_{ab,0}$, and $\Delta h_{ab} = h_{ab,1} - h_{ab,0}$.[27]

The purpose of this study was to evaluate the changes in color, by means of visual evaluation and use of a colorimeter, of four whitening agents used at home with customized trays for one hour per day over the course of 14 days to show which concentration is the most effective and to evaluate the sensitivity produced.

MATERIALS AND METHODS

This was a controlled, parallel, randomized, one-center clinical study undertaken at the Faculty of Medicine and Dentistry in Santiago de Compostela (Spain) that was approved by the faculty's ethics committee. Ninety-six volunteers participated, 68 women and 28 men, with an average age of 25.9 years (±5.6 years), selected consecutively according to the inclusion and exclusion criteria described in Table 1. Before beginning the treatment, all of the participants signed an informed consent in which the treatment they were going to receive, as well as its side effects, were explained in detail. They were randomly divided into four study groups of 24 individuals by alphabetical order. Each of the groups used the following outpatient whitening gels: 10% CP (Opalescence PF 10%, Ultradent, South Jordan, UT, USA); 15% CP (Illuminé Home, Dentsply, Konstanz, Germany); 7.5% HP (Poladay, SDI, Melbourne, Australia); and 9.5% HP (Poladay, SDI).

At the first visit, personal information was recorded and a dental prophylaxis (Kerr Hawe Cleanic, Kerr Hawe SA, Bioggio, Switzerland) was performed for all patients in order to facilitate extrinsic stain removal. Immediately, alginate impressions (Cavex, Fast Set, Dust Free, Keur & Sneltjes, The Netherlands) were made of both arches. After obtaining models cut in a horseshoe shape, customized trays without reservoirs were made using 1-mm-thick soft trays (Clear-Mouthguard Henry Schein Inc, Melville, NY, USA) with an Econo-Vac machine (Buffalo Dental Manufacturing, Syosset, NY, USA). The splints were then trimmed to within 1 mm of the gingival margin, taking care not to cover the interdental papillae. A positioner for the colorimeter was also made using 4-mm-thick

Table 1: *Inclusion and Exclusion Criteria*

Inclusion Criteria	Exclusion Criteria
18 y of age or older	Systematic illness; persons undergoing medical treatment
A minimum of 24 natural teeth, including incisors, canines, and premolars in both arches	Requirement of antibiotic prophylaxis for dental treatment
Good oral hygiene	Patients under analgesic and/or anti-inflammatory therapy
Absence of periodontal disease	Pregnant or breast-feeding women
Sillness and Löe index $\leq$1	Tumors of the soft or hard tissues of the oral cavity
Absence of gingival recession	Xerostomia; alterations of the oral mucosa
Availability in the area of the study for a minimum of 4 wk	Smokers
	The presence of restorations in the six anterior teeth of either arch
	Active caries
	Periapical pathology
	Staining due to tetracycline or fluorosis
	Structural alteration of the tooth structure; Amelogenesis
	Exposed dentin in anterior incisors; general hypersensitivity
	Bruxism
	The use of fluoride supplementation or desensitizing agent
	The use of stain-inducing medications for oral use
	Removable prosthesis
	Currently undergoing orthodontic treatment
	Having undergone any other previous whitening treatment

clear plates. Four orifices were made in the middle third of the maxillary central incisors and canines of the same diameter at the point of the colorimeter's probe with a 6-mm external diameter trephine in order to ensure that the color was always recorded in the same place on the tooth (Figure 1).

The colorimeter used was a Vita Easy Shade (Vita Zahnfabrik, Bad Säckingen, Germany). It is designed for dental use and has a 6-mm-diameter sensor. This colorimeter registers the colors of the Vita Classical Guide and shows the L*, a*, b*, C*, and h* coordinates of the chromatic space of the measurement taken.

Two examiners received training through color evaluation of 42 dental students. Measurements were taken in the middle third of the maxillary right central incisor. The room in which the evaluations were made had an illumination of 6.500 Kelvin. A shade guide (Vitapan Classical, Vita Zahnfabrik) was used and ordered by lightness according to the manufacturer's recommendations. The training was completed when an 80% concordance between both observers was reached by means of the kappa test.

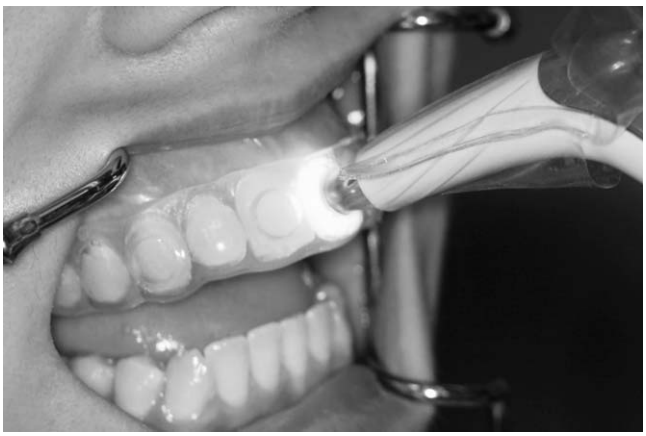

Figure 1. *Positioner for the digital spectrophotometer.*

During the second visit, the tray and position-finder for the colorimeter of each participant were tested for fit. Each participant was given a whitening kit. In all of the groups, the administration of the gel was for one hour per day over the course of two weeks. Before beginning the treatment, the two observers determined the initial dental color of the middle third of the upper right-hand central incisor using the Vitapan Classical shade guide, ordered by lightness. To determine the clinical result and for statistical analysis, a numeric value between 1 and 16 (shade tabs) corresponding to the sequence: B1 (1), A1 (2), B2 (3), D2 (4), A2 (5), C1 (6), C2 (7), D4 (8), A3 (9), D3 (10), B3 (11), A3.5 (12), B4 (13), C3 (14), A4 (15), C4 (16), and C4 (16) was assigned.[10,19,21,22] For example, if after the whitening treatment the color changed from A2 to a B1, it was counted as a change of four shade tabs.

With the position-finder placed in the mouth, the Vita Easyshade colorimeter was used to measure the Vita Classical colors and L*, a*, b*, C*, h* coordinates for the right and left maxillary central incisors and canines.

The subjects registered the sensitivity they experienced during the whitening treatment on a daily basis, filling in a questionnaire with a simplified

Table 2: *The Mean (Standard Deviation, SD) of CIE L*a*b* and L*C*h* Baseline Values Measured by the Colorimeter in 96 Participants*

	Maxillary Central Incisors (n=192), Mean (SD)	Maxillary Canines (n=192), Mean (SD)	*p*-Value Incisors-Canines
L*	83.5 (4.2)	77.4 (4.3)	<0.001***
a*	−1.1 (0.9)	1.6 (1.2)	<0.001***
b*	18.7 (3.5)	27.4 (3.9)	<0.001***
C*	18.8 (3.4)	27.6 (4.0)	<0.001***
h*	93.8 (3.3)	86.6 (3.1)	<0.001***

*** Statistically significant difference at 0.1% level.

scale, as follows: 0 = none; 1 = mild; 2 = moderate; 3 = considerable; and 4 = severe.[10,19] When a participant had differing severities of sensitivity in the first or second week, the highest value was registered.

At the following visit, 14 days later, and after having completed the treatment, the color was determined both by the observers and the colorimeter, and the sensitivity survey was collected.

The assumption of normality for all variables was analyzed by means of the Kolmogorov-Smirnov test. For the study of the measurement of the observers and the colorimeter via the Classical Guide, the marginal homogeneity test was used. The study of the CIE Lab parameters was conducted by means of the Wilcoxon test. The Kruskal-Wallis and Friedman tests were utilized for the study of the sensitivity registered with the different products each day.

RESULTS

All 96 participants completed the study. The determination of color made by the observers in the middle third of the upper central incisor of the participants before beginning the treatment was, according to the Vita Classical Guide shade tabs, 5.33 (±3.09), whereas the color registered by the colorimeter was 4.20 (±2.89). This difference was statistically significant ($p<0.01$). In the measurements made with the colorimeter at the baseline in both the central incisors and upper canines, there were significant differences in all the CIE L*a*b* and CIE L*C*h* parameters ($p<0.001$). The canines had a lower lightness and more chromatic intensity or saturation. Their positive values for a* (1.6 ± 1.2) indicate a redder color than was associated with the incisors. The value h* was superior in the incisors (93.8 ± 3.1) to that in the canines (86.6 ± 3.1), which indicates that the incisors had a more yellow color. This was also indicated by the higher positive value for b* (Table 2).

The color changes noticed by the observers and the colorimeter in the right upper central incisor after 14 days of treatment were significant in all of the groups ($p<0.01$). If the colors noted by the observers are compared with the data from the colorimeter, there were significant differences between the two groups (Table 3). With regard to the CIE L*a*b* parameters, in all the groups, there were significant differences noted when comparing the recordings at the beginning and the end of the treatment (Table 4). In all of the gels studied, on the 14th day, when the whitening treatment was stopped, the teeth had higher luminosity (L*) and lower chromatic intensity (C*). The angle of the value for the color (h*) increased, which indicates a movement from yellow toward green. The decreases in the a* and b* values indicate movement toward green and blue, respectively. No statistically significant differences in ΔL* and ΔE* were detected between the groups. Once the whitening had been completed, the differences in the results obtained between incisors and canines were statistically significant in all of the groups and for all of the parameters, with the color change in the canines being most noticeable (Table 5; Figure 2).

With regard to the sensitivity reported by the patients, no differences were found in the numbers of patients who reported sensitivity, during the first

Table 3: *Mean (Standard Deviation, SD) Changes in Color Values of the Middle One-third of the Facial Surface of the Right Upper Central Incisor After 14 Days, as Measured by Examiners (Visual Evaluation) and by Spectrophotometer; Vita Classical Shade Tabs*

	Examiners (n=96), Mean (SD)	Spectrophotometer (n=96), Mean (SD)	*p*-Value examiners-spectrophotometer
CP 10%	3.5 (2.2)	2.0 (1.7)	<0.05*
CP 15%	1.1 (0.7)	0.7 (0.9)	0.230
HP 7.5%	4.9 (2.3)	1.3 (1.9)	<0.001***
HP 10%	1.8 (1.6)	1.0 (2.0)	0.176

Abbreviations: CP, carbamide peroxide; HP, hydrogen peroxide.
*** Statistically significant difference at 0.1% level; * Statistically significant difference at 5% level.*

Table 4: *Mean (Standard Deviation, SD) Changes in Tooth Color Values Measured by Spectrophotometer in Both Maxillary Central Incisors and Canines After Whitening (14 Days)*

Group	Mean (SD)	*P*, 0-14 d
CP 10% (n=24)		
ΔL*	4.4 (2.8)	<0.001***
Δa*	−1.0 (1.2)	<0.001***
Δb*	−4.3 (2.8)	<0.001***
ΔC*	−4.4 (3.2)	<0.001***
Δh*	1.6 (2.4)	<0.001***
ΔE*	6.6 (3.5)	<0.001***
CP 16% (n=24)		
ΔL*	3.7 (3.8)	<0.001***
Δa*	−1.2 (1.2)	<0.001***
Δb*	−3.8 (3.6)	<0.001***
ΔC*	−3.7 (3.5)	<0.001***
Δh*	4.3 (3.6)	<0.001***
ΔE*	6.5 (4.0)	<0.001***
HP 7.5% (n=24)		
ΔL*	3.4 (3.7)	<0.001***
Δa*	−1.7 (1.3)	<0.001***
Δb*	−5.3 (3.3)	<0.001***
ΔC*	−5.5 (2.9)	<0.001***
Δh*	5.9 (3.4)	<0.001***
ΔE*	7.4 (2.6)	<0.001***
HP 10% (n=24)		
ΔL*	3.6 (3.0)	<0.001***
Δa*	−1.3 (1.4)	<0.001***
Δb*	−4.4 (4.6)	<0.001***
ΔC*	−4.4 (4.5)	<0.001***
Δh*	4.8 (3.9)	<0.001***
ΔE*	7.1 (4.0)	<0.001***

Abbreviations: CP, carbamide peroxide; HP, hydrogen peroxide.
**** Statistically significant difference at 0.1% level.*

Table 5: *Mean (Standard Deviation, SD) Differences in Color Between Central Incisors and Canines After Whitening; Measurements Taken in Both Upper Central Incisors and Canines*

Group	Maxillary Central Incisors (n=48), Mean (SD)	Maxillary Canines (n=48), Mean (SD)	*p*-Value Incisors-Canines
CP 10%			
ΔL*	2.7 (1.7)	6.0 (2.8)	<0.001***
Δa*	−0.1 (0.8)	−2.0 (0.9)	<0.001***
Δb*	−2.5 (1.7)	−6.1 (2.5)	<0.05*
ΔC*	−2.7 (3.1)	−6.1 (2.3)	<0.05*
Δh*	0.2 (2.0)	6.5 (3.3)	<0.001***
ΔE*	4.1 (1.8)	9.1 (3.1)	<0.001***
CP 16%			
ΔL*	1.9 (2.5)	5.5 (4.0)	<0.001***
Δa*	−0.2 (0.5)	−2.1 (1.0)	<0.001***
Δb*	−1.9 (2.7)	−5.8 (3.3)	<0.001***
ΔC*	−1.9 (2.4)	−5.4 (3.5)	<0.001***
Δh*	2.1 (2.5)	6.5 (3.3)	<0.001***
ΔE*	4.0 (2.3)	9.1 (3.8)	<0.001***
HP 7.5%			
ΔL*	1.2 (2.6)	5.5 (3.5)	<0.001***
Δa*	−0.7 (0.5)	−2.7 (1.2)	<0.001***
Δb*	−3.9 (2.0)	−6.7 (3.7)	<0.001***
ΔC*	−4.0 (1.6)	−7.0 (3.2)	<0.001***
Δh*	4.8 (2.5)	7.0 (3.9)	<0.01**
ΔE*	5.0 (1.8)	9.8 (3.5)	<0.001***
HP 10%			
ΔL*	1.8 (1.6)	5.5 (3.1)	<0.001***
Δa*	−0.4 (0.7)	−2.3 (1.3)	<0.001***
Δb*	−3.3 (3.0)	−5.6 (5.5)	<0.001***
ΔC*	−3.1 (3.0)	−5.7 (5.4)	<0.001***
Δh*	3.2 (3.9)	6.5 (3.3)	<0.001***
ΔE*	4.6 (2.3)	9.7 (3.8)	<0.001***

Abbreviations: CP, carbamide peroxide; HP, hydrogen peroxide.
**** Statistically significant difference at 0.1% level; ** Statistically significant difference at 1% level; * Statistically significant difference at 5% level.*

week (p=0.381), the second (p=0.103) week, or during the 14 days of treatment (p=0.202). There were no differences in the intensity of the sensitivity (Table 6).

DISCUSSION

The whitening trays were not made with reservoirs. Some researchers claim that the use of reservoirs does not produce improved whitening[28] or that they result in whitening that is only appreciable by a colorimeter and not with color guides or photographs.[29] Kirsten and others[30] affirm that the reservoirs result in an increase in inflammation in the gingival mucosa.

The subjectivity of the evaluation of whitening by observers has already been described.[2,31,32] Furthermore, the classic Vita guide was not designed for judging the change in color after whitening. The guide is also nonlinear in scale and lacks color uniformity, while the overlap between similar colors provides little resemblance to reality.[10,33] One explanation for observers seeing darker colors than the colorimeter at the beginning of the study and lighter ones at the end could be because the study was not blinded. After undergoing the whitening treatment in the evaluations using the Vita Classical Guide ordered by lightness, we observed that in

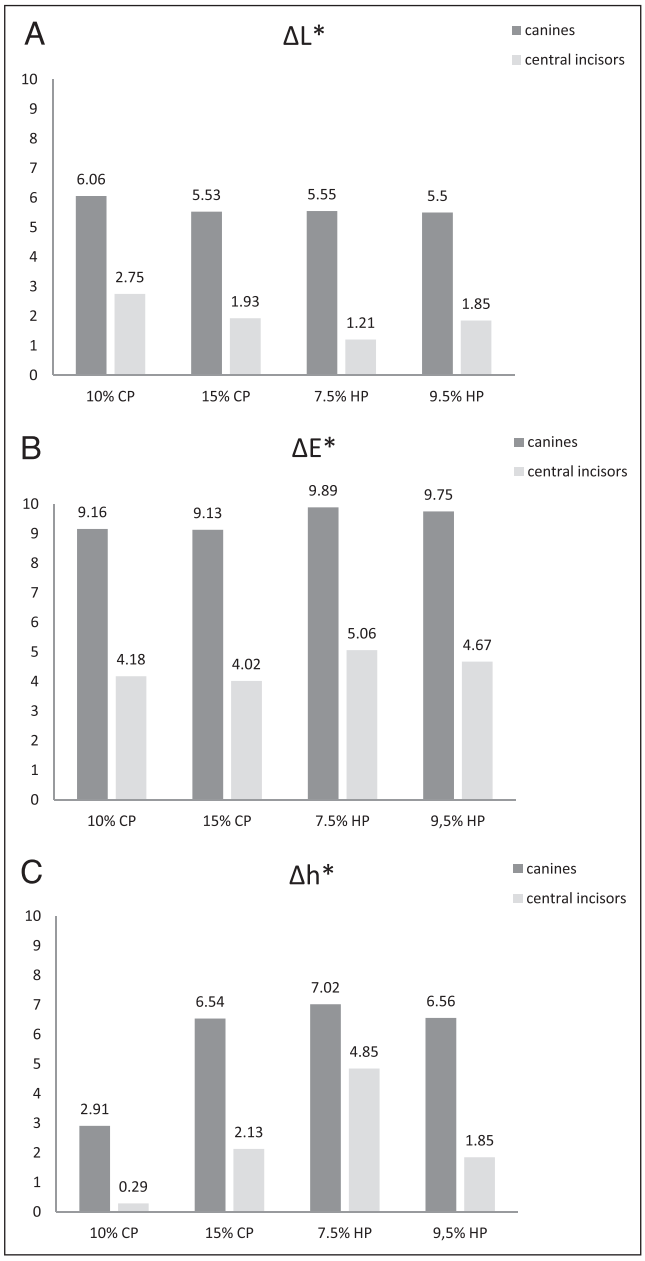

Figure 2. *(A) Mean values of the changes in lightness (ΔL*) measured with the colorimeter in upper central incisors and canines after 14 days of treatment. (B) Mean values of the changes in ΔE* measured with the colorimeter in upper central incisors and canines after 14 days of treatment. (C) Mean values of the changes in value or tone (h*) measured with the colorimeter in upper central incisors and canines after 14 days of treatment.*

some cases, the color was considered by the observers to be even lighter than B1, a lightness score that could not be quantified. In similar studies in which 10% CP was used, observers determined with the Vita Classical Guide that there had been whitening of 5.4[10] and 3.85 shade tabs.[23] In this study, the whitening measured 3.59 shade tabs.

In the majority of the studies on the subject, positioners were not designed to ensure that the colorimeter would always measure the color at the same place.[19,21,34] The teeth in which the measurements were made varied according to the study, with measurements occurring in the upper central incisors,[10,24] the upper lateral and central incisors,[15,18] in the six anterior maxillary teeth,[13,16,19,21,22,25] or in the upper and lower anterior teeth.[20] In this study and in others[35] it has been determined that, between nonwhitened incisors and canines, there are statistically significant differences in the CIE L*a*b* values. Additionally, it has been affirmed that the data obtained by different colorimeters are not comparable.[36-39] When software for the analysis of images obtained by a photographic camera is used to measure the degree of whitening, it is always different.[22-25]

Meireles and colleagues[19] used the same colorimeter to measure the color in the six anterior teeth after a two-hour daily application (over the course of three weeks) of 10% CP: $\Delta L^* = 3.8$, $\Delta E^* = 4.3$ and 16% CP $\Delta L^* = 3.7$, $\Delta E^* = 4.6$. For the same concentrations of whitening agent, our results, $\Delta L^* = 4.41$ and 3.73 and $\Delta E^* = 6.67$ and 6.57, were measured in central incisors and canines, respectively. Using a Vita Easyshade Compact colorimeter for a 10% CP treatment applied for one hour per day over the course of 16 days, Cardoso and colleagues[10] measured $\Delta E^* = 5.8$ in the upper central incisor. Our result for 10% CP in the incisor measurements was $\Delta E^* = 4.18$.

Studies in which different concentrations of CP (10%, 15%-17%) are compared have concluded that the whitening results are similar[18-20] or, on the contrary, that higher concentrations whiten more.[40] Delgado and colleagues[16] affirm that there are no differences between 9% HP and 20% CP. Comparing 7.5% HP and 20% CP, Ziebolz and colleagues[24] did not see differences in ΔL^* and Δa^*. This finding coincides with our results, in which no significant differences were found in ΔL^* and ΔE^* in the study groups.

The evaluation of sensitivity is reported by patients on a scale that is different in each study: "Yes" or "No,"[15] from 0 (none) to 4 (severe),[10,19,40] and from 0 to 10 (high hypersensitivity).[24] In those studies[19,24,25,40] in which a scale similar to the one used in this work was used, the average intensity of the sensitivity provoked by at-home whitening, as in this study, was never "considerable" or "severe."

Table 6: *Patients Experiencing Sensitivity and its Intensity; Percentages in Parentheses; Mean (Standard Deviation)*[a]

	CP 10% (n=24)	CP 15% (n=24)	HP 7.5% (n=24)	HP 9.5% (n=24)
Sensitivity days 1-7	12 (50%)	11 (45.8%)	13 (54.2%)	13 (54.2%)
Intensity of sensitivity days 1-7	0.33 (0.47)	0.38 (0.49)	0.36 (0.50)	0.53 (0.64)
Sensitivity days 8-14	6 (25%)	11 (45.8%)	10 (41.7%)	11 (45.8%)
Intensity of sensitivity days 8-14	0.20 (0.42)	0.29 (0.46)	0.35 (0.48)	0.38 (0.52)
Sensitivity days 1-14	13 (54.2%)	13 (54.2%)	14 (58.3%)	14 (58.3%)
Intensity of sensitivity days 1-14	0.27 (0.46)	0.33 (0.47)	0.37 (0.51)	0.44 (0.57)

Abbreviations: CP, carbamide peroxide; HP, hydrogen peroxide.
[a] *Tooth sensitivity evaluation: 0 = no; 1 = mild; 2 = moderate; 3 = considerable; and 4 = severe.*

In other works, the percentages of participants who had sensitivity with the 10% CP varies from 13%, with a one-hour daily application,[10] to 41% and 43% for 16% CP applied for two hours per day.[19] In our study, the figure was 54% for a one-hour daily application for both concentrations. With the 7.5% HP, the sensitivity was 44%[24] and 58% in the present study. We registered 58% of patients reporting sensitivity with the 9.5% HP, whereas in another study in which 9.5% HP was applied for 30 minutes daily over the course of nine days, it did not surpass 30%. Meireles and colleagues[19] affirm that 16% CP provokes more sensitivity than does 10% CP. Ziebolz and colleagues[24] maintain that more sensitivity occurs with 20% CP than with 7.5% HP, whereas in another study,[40] as in the present work, there were no significant differences between these concentrations. Furthermore, the CP gels used in this study used desensitizing products in their composition.

CONCLUSIONS

Based on the results obtained in this study, in which no statistical differences in the degree of whitening with the different gels were found, we would recommend the use of gels at a lower concentration, as is recommended by the American Dental Association and the Scientific Committee on Consumer Products of the European Commission. As a result of the use of different colorimeters or photographic cameras with measurements taken on different teeth, we believe that studies evaluating the effectiveness of whitening treatments are difficult to compare results. A standardized method is needed for the evaluation of the effectiveness of whitening and the safety of gels applied at home. All of the products used produced a lowering of chroma or saturation and an increase in lightness and hue. Negative a* and b* values indicate a movement in the chromatic space toward green and blue, respectively. These changes were more noticeable in the canines.

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

Impact of Quantity of Resin, C-factor, and Geometry on Resin Composite Polymerization Shrinkage Stress in Class V Restorations

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Clinical Relevance

Adhesive dentistry allows for the simple removal of decayed tissue to guide preparation design. Knowledge about differences in stress concentration within cavities can help in understanding the impact of shape and cavosurface angle of the cavity, optimizing the distribution of stress during the cure of the restorative material and improving the expected lifetime of the restoration.

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SUMMARY

Objective: This study evaluated the effect of quantity of resin composite, C-factor, and geometry in Class V restorations on shrinkage stress after bulk fill insertion of resin using two-dimensional finite element analysis.

Methods: An image of a buccolingual longitudinal plane in the middle of an upper first premolar and supporting tissues was used for modeling 10 groups: cylindrical cavity, erosion, and abfraction lesions with the same C-factor (1.57), a second cylindrical cavity and abfraction lesion with the same quantity of resin (QR) as the erosion lesion, and then all repeated with a bevel on the occlusal cavosurface angle. The 10 groups were imported into Ansys 13.0 for two-dimensional finite element analysis. The mesh was built with 30,000 triangle and square elements of 0.1 mm in length for

all the models. All materials were considered isotropic, homogeneous, elastic, and linear, and the resin composite shrinkage was simulated by thermal analogy. The maximum principal (MPS) and von Mises stresses (VMS) were analyzed for comparing the behavior of the groups.

Results: Different values of angles for the cavosurface margin in enamel and dentin were obtained for all groups and the higher the angle, the lower the stress concentration. When the groups with the same C-factor and QR were compared, the erosion shape cavity showed the highest MPS and VMS values, and abfraction shape, the lowest. A cavosurface bevel decreased the stress values on the occlusal margin. The geometry factor overcame the effects of C-factor and QR in some situations.

Conclusion: Within the limitations of the current methodology, it is possible to conclude that the combination of all variables studied influences the stress, but the geometry is the most important factor to be considered by the operator.

INTRODUCTION

Besides the fracture of remaining tooth structure, some effects of microleakage, such as stained and degraded margins and secondary caries, are common causes of failure of resin composite restorations in clinical practice. If the material is rigid, the shrinkage of the composite can induce stress on adhesive interfaces that mechanically challenge the hybrid layer and potentially overcome the bond strength at the interface.[1,2] Gaps in the interface can allow marginal leakage, followed by discoloration and bacterial contamination. The association of secondary caries and marginal staining with failures of the adhesive interface would be rational, in spite of the absence of validating clinical studies.[3-5]

Shrinkage of dental resin composites occurs due to the addition of monomer molecules into a polymer network,[6] reducing the space among the original molecules. This chemical reaction generates contraction stresses in the resin composite with deformation of the surrounding tooth structure.[7] Besides the polymerization reaction, other factors can influence the shrinkage stresses and gap formation at the tooth-restoration interface.[1]

Since microleakage can lead to clinical restoration failures, a good marginal adaptation might increase the service life of a resin composite. The quality of the marginal adaptation can be influenced by the bonding system and by factors related to the stress developed during the polymerization of the material. Stress is influenced by factors such as the mechanical properties and amount of shrinkage of the resin composite, the cavity size and geometry, and the restorative placement and curing technique.[8]

The C-factor (CF) is defined as the ratio of bonded to unbonded surfaces of the dental cavity, and its value is supposed to be directly related to the stress developed at the interface bonding area.[9,10] Several laboratory studies with tensilometers have shown a positive correlation between shrinkage stress and CF,[11-13] but clinical assessments need to be carefully performed due to the complex geometries involved. Stresses generated by a composite bonded within a cavity depend not only on the CF but also on the compliance of the remaining wall structures and the mass or volume of resin composite involved.[14]

Many techniques and materials have been developed in an attempt to obtain long-term retention for esthetic restorations placed in cervical areas. For moderately large-sized restorations, incremental resin composite placement is recommended to decrease the effects of polymerization shrinkage. However, the incremental technique also has disadvantages, such as the possibility of incorporating voids between composite layers and the increased time required to place and cure each layer. This has encouraged the development of composites that report adequate polymerization with a 4-mm depth, allowing for a bulk fill. A previous study has suggested that fewer increments or a bulk-fill could be successful.[15] The present study approaches this idea conceptually by investigating the impact of different cavity designs on the shrinkage polymerization stress developed by the resin composite.

A finite element analysis (FEA) was applied to factors inherent to shrinkage stress. The purpose of this study was to evaluate the effect of quantity of resin (QR), C-factor, and geometry on shrinkage stress of Class V restorations simulating a bulk-fill insertion technique. The null hypotheses tested were that the QR, C-factor, geometry, and existence of a bevel have no influence on the polymerization shrinkage stress in Class V resin composite restorations.[16-20]

MATERIALS AND METHODS

An image of a buccolingual longitudinal plane of an upper first premolar was replicated in the CAD (Computer Aided Design) Rhinoceros software (ver-

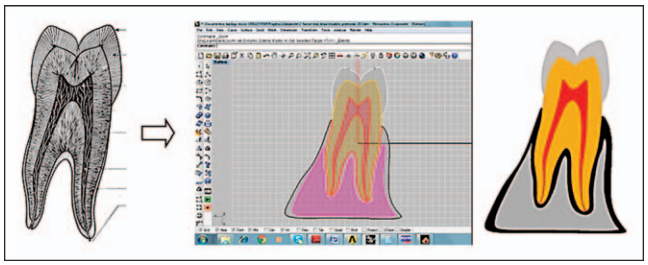

Figure 1. *Two-dimensional geometry of a healthy upper first premolar built in CAD software.*

sion 4.0 SR8, McNeel North America, Seattle, WA, USA) and virtually inserted into an image of the alveolus of the posterior maxillary alveolar process obtained from a human anatomy book [21] (Figure 1). A healthy tooth was used as the standard for all groups.

The first situation, model 1, was a Class V erosion lesion, measuring 3 mm gingivo-occlusally and 2 mm in depth, and simulated the preparation made by a 3-mm diameter spherical bur. The values of C-factor (1.57) and cross-sectional area of restorative material (3.4 mm^2) obtained for this model were used as a reference for further models. The cross-sectional area represents the QR in this two-dimensional analysis. Models 2-5 used abfraction and cylindrical geometries, first holding the C-factor constant to model 1 and then the cross-sectional area. Each of these five models was further modified by inclusion of an occlusal cavosurface marginal bevel at 158° and 1-mm length to analyze the effect of the bevel on the shrinkage stress distribution. Figure 2 shows the 10 groups analyzed in this work.

These CAD models were imported as parasolid format files into ANSYS software (ANSYS 13.0, ANSYS Inc, Houston, TX, USA) for the numerical simulations by FEA. All materials were considered homogenous, linearly elastic, and isotropic. Their mechanical properties are summarized in Table 1.

The mesh was built with triangle and square elements with slow transition and high smoothing as mesh controls. Tests varying the size of elements were carried out until 10% of convergence of the results was reached, which determined that the ideal element size was 0.1 mm. The total number of elements was about 30,000 in all models.

The restoration-tooth interfaces in all the models were considered perfectly bonded. The polymerization shrinkage of the resin composite was simulated by thermal analogy: the initial temperature was reduced by 1°C and, by attributing a coefficient of

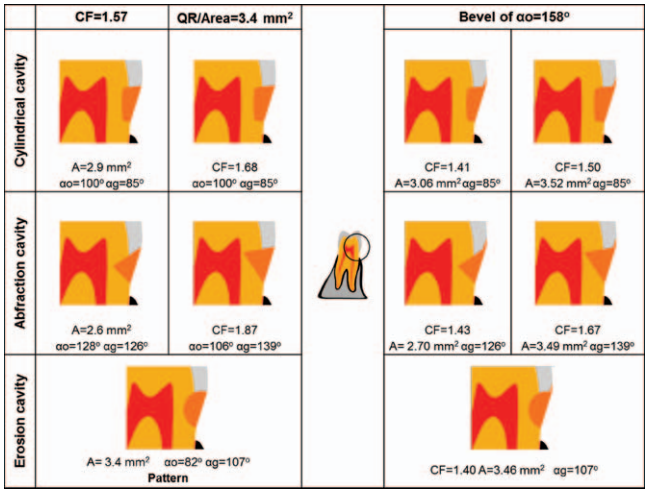

Figure 2. *Geometry of the 10 cavities studied according CF, cross-sectional area of QR, and the angle of cavosurface margin (αo=occlusal and αg=gingival).*

thermal expansion of 0.0021379/°C to the resin, a 0.64% volumetric shrinkage was experienced. The nodes of the top line of the maxillary cortical bone were fully constrained in all directions.

A linear static structural analysis was performed to calculate the stress distribution in the different restoration configurations. The von Mises stress (VMS) was used to observe whether the results were coherent with what should be expected in such biomechanical situations (related to the coherence of the numerical simulations) and to observe the dissipation of the distortional energy throughout the materials. Due to tooth tissue exhibiting a relatively brittle behavior, the maximum principal stress (MPS) was chosen to analyze the stress concentration areas in the occlusal and gingival cavosurface angle regions.

RESULTS

The influence of CF, QR, and bevel on equivalent von Mises and maximum principal stresses is shown in Figures 3 and 4, respectively. The symbol "α" represents the angle between the cavosurface margin and occlusal (αo) and gingival (αg) inner cavity walls. As indicated by the scales, the hotter (more red) colors in the figures represent higher values of stress, in MPa.

The numbers found in the rows labeled VMS-MPa or MPS-MPa represent the stress on the occlusal margin (left cell) and the gingival margin (right cell). In Figure 3, when there are two numbers for VMS in the same cell, the higher value refers to the peak

Table 1: *Mechanical Properties of the Material Used in the Numerical Simulations*

Material	Elastic Modulus, GPa	Poisson's Ratio	Coefficient of Thermal Expansion, mm/°C; Reference Temperature: 25°C	Reference
Enamel	41.00	0.30	—	Ko and others, 1992[16]
Dentin	18.6	0.31	—	Rees and others, 1994[17]
Pulp chamber	0.002	0.45	—	Rubin and others, 1983[18]
Cortical bone	13.7	0.30	—	Ko and others, 1992[16]
Cancellous bone	1.37	0.30	—	Ko and others, 1992[16]
Periodontal ligament	0.069	0.45	—	Holmes and others, 1996[19]
Low shrinkage composite	6.00	0.30	0.0021379 (for 0.64% of pos gel volumetric shrinkage)	Boaro & others, 2010[20]

stress that occurred distant to the restoration edge, and the lower value to the stress developed near the edge.

It can be observed in Figure 3 that the presence of a bevel resulted in a decrease of the VMS on the cavosurface margin on enamel.

The highest VMS in the abfraction geometry was concentrated along the pulpal surface, and its value is shown below the respective image.

For geometries with the same CF (the first column of Figures 3 and 4), the highest values of peak VMS and MPS were erosion (42.90 and 36.14 MPa), cylindrical (32.45 and 26.96 MPa), and abfraction (18.54 and 16.05 MPa). For the factor QR, this followed the sequence of 3.4 mm^2 (erosion), 2.9 mm^2 (cylindrical), and 2.6 mm^2 (abfraction).

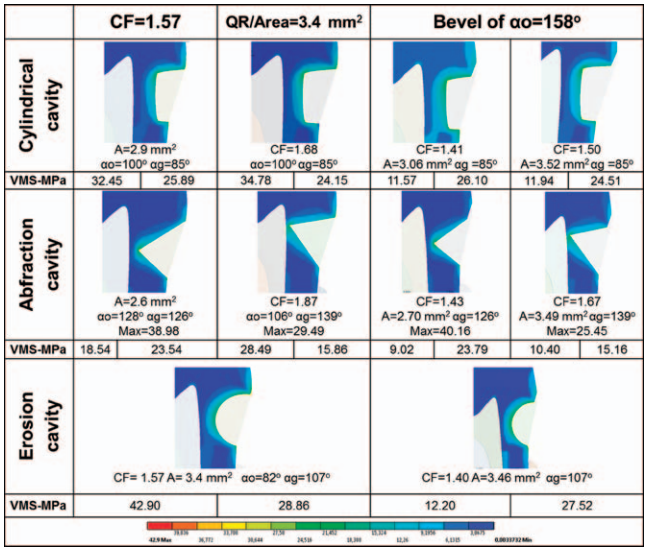

Figure 3. *Details of equivalent von Mises stress of three types of cavities with the same CF, the same cross-sectional area of QR, and with a bevel.*

In the second column of Figures 3 and 4, for groups with the same QR, the order of peak VMS and MPS was erosion (42.90 and 36.14 MPa), cylindrical (34.78 and 28.18 MPa), and abfraction (28.49 and 22.52 MPa). This was similar to that seen with the three geometries having the same CF as shown in the first column. However, in this case, the sequence did not correlate with the CF of 1.87 for abfraction, 1.68 for cylindrical, and 1.57 for erosion. Instead, the cavity with the lower CF showed the higher MPS and VMS values.

In the beveled cavities (the third and fourth columns of Figures 3 and 4), the values of MPS for the cavosurface margin were similar, ranging from 8.48 to 11.83 MPa, as the CF ranged from 1.40 to 1.67, and the area (QR) from 2.7 to 3.46 mm^2, ie, the bevel tended to equalize the values for all the cavities. The bevel decreased the peak stress on the

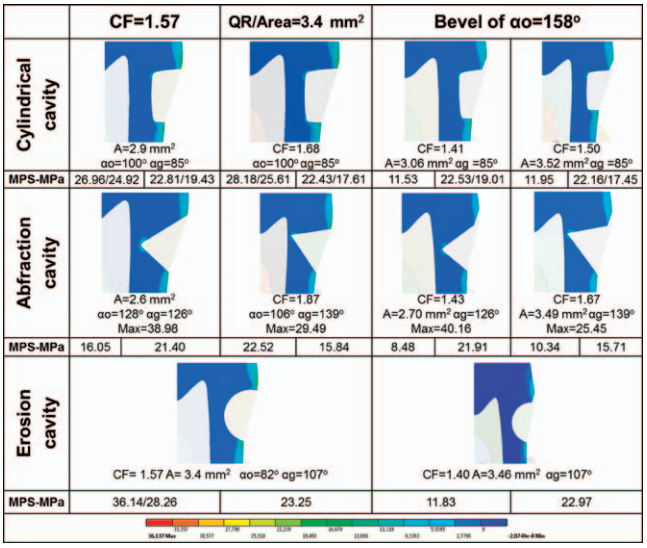

Figure 4. *Details of MPS of three types of cavities with the same CF, the same cross-sectional area of QR, and with a bevel.*

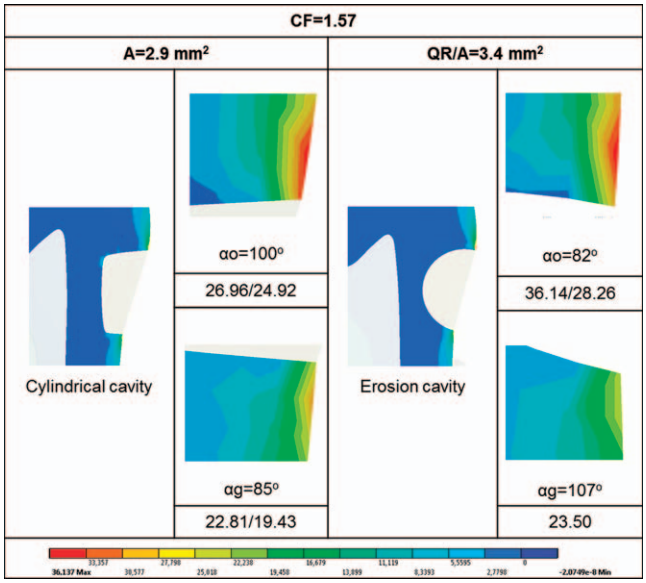

Figure 5. *Difference among the MPS fields in the occlusal and gingival cavosurface angles of the erosion and cylindrical cavities without bevel.*

enamel wall, but had no effect on the gingival wall for all geometries. For erosion and cylindrical unbeveled geometries, the peak stress was close to the cavosurface margin and, when beveled, the overall peak stress decreased. For the abfraction geometry, the peak stress was always more concentrated in the inner angle of the cavity, and the bevel did not have a significant effect. The reduction of the overall peak stress in the entire cavity by placing a bevel was more pronounced in the erosion and cylindrical geometries.

In the four models of abfraction, those presenting a higher CF (Figures 3 and 4) showed the lowest peak stress.

The bevel changed the cavosurface angles; Figure 5 shows its influence on the peak stress distribution in the cavities where there were reductions in the overall peak stress. Obtuse angles in the cylindrical geometry ($\alpha o = 100°$) showed lower values of peak stress (26.96 MPa) than the acute angle (36.14 MPa) in the erosion geometry ($\alpha o = 82°$).

DISCUSSION

The present study used FEA to investigate the impact of cavity designs on the polymerization stress distribution of direct resin composite restorations in Class V preparations. The results showed a significant influence of the geometry, volume, and C-factor on the shrinkage stress after curing a simulated microhybrid bulk-filled composite.

The two-dimensional models correspond to a simplification of three-dimensional structures. They are not a fully representative simulation of a complex three-dimensional structure, such as the real tooth, because they simulate a condition where a structure has a constant shape along its thickness. The extrapolation of what occurs within these simplified geometries cannot be made directly to the corresponding actual types of cavities, which have shape variation along the mesiodistal thickness. The results represent, partially, what occurs in the buccolingual midline of the tooth crown. However, this simplification demonstrates the importance of considering variables such as the amount of resin that shrinks, CF, and cavity geometry on stresses generated during the curing process. Care is needed in interpreting the two-dimensional simulations: the CF was calculated using the two-dimensional lengths of bonded interface and free surface, while the QR was calculated as an area rather than volume.

There is great difficulty in representing, computationally, the material properties, boundary conditions and loading faithful to the conditions found in clinical situations,[22] and these simplifications mean that the computational simulation will not be absolutely faithful to a real-life model that represents the structures, materials, and dental tissues *in vivo*.[23]

With regard to composite resins, shrinkage stress is a problem that has been investigated by many, since it is considered a significant contributor to the clinical failure of direct adhesive restorations. Many discussions have taken place about the variables that most influence this stress. According to previous studies, filler volume fraction,[24] polymerization shrinkage,[25] camphorquinone concentration,[26] amine concentration,[27] specimen geometry,[28] and curing method[29] are among many factors considered significant in the development of polymerization shrinkage stresses.

With FEA, it is possible to isolate the variables of interest (eg, CF, geometry, volume, area, angle) and to study their individual or combined effects in cases where it is not possible or it is too difficult to perform in an *in vitro* experiment. We simulated two types of lesions with three different geometries: clinical, noncarious lesions (abfraction and erosion), and a standard preparation for microleakage laboratory tests (cylindrical) along with three other variables: C-factor, area (representing composite quantity, QR), and bevel. The color gradient allows for qualitative/quantitative comparison among the

groups. In the first three groups, we standardized the C-factor and, because of the geometry, the area (QR) was different. That was the greatest factor impacting wall cavity stress, so using the erosion group, the area (QR) was standardized to observe the effect of geometry on stress concentration.

Many evaluations about which material or restoration technique is most suitable for Class V restorations are made by microleakage tests, with circumferential or cylindrical cavities. However, clinically, the walls in this region do not always have the geometric shape of the cavities used in microleakage laboratory tests. The chosen variables were analyzed to determine whether it is appropriate to extrapolate the results of laboratory Class V adhesive tests made with cylindrical cavities to cases of erosion or abfraction and to make the operator aware of design variables one can control to reduce the stress concentration.

Figures 3 and 4 show the same behavior for the two standardized situations: C-factor and area (QR) and the impact of geometry can be observed. The erosion geometry was made as a hemisphere because, according to the hypothesis idealized, the higher the angle of restorative material at the enamel margin, the higher the stress concentration. Thus, the worst-case scenario would be close to the 90° created by a spherical bur.

There were two different behaviors observed in this study: first, the higher the angle formed at the cavosurface margin, the lower the VMS (Figure 3) and MPS (Figure 4), except in the cylindrical cavity, where the geometry changed the isocurve distribution[1] and, second, the greatest stress was not located on the edge (Figure 5).

Figure 5 shows that the cavosurface angle influences the value of the tensile stress, the smaller the angle of tooth structure, the greater the stress concentration. For this reason, it is possible to observe in Figures 3 and 4 that the field pattern for VMS and MPS in cylindrical and erosion cavities are more similar to each other (with the closest cavosurface angle values) and different from the abfraction lesions.

Shrinkage polymerization stress has been related to many factors, such as type of composite, light source, energy density, elastic modulus, degree of conversion, C-factor, geometry, and anatomic region of the cavity.[30] The transmission of the stress from one structure to another depends not only on their mechanical properties, but also on the relationship between them, ie, the boundary conditions. The CF has the potential to impact the plastic deformation and, thus, the relaxation of the material occurring during polymerization.[8,11] Figures 3 and 4 show geometry and area overcoming the CF effects: when the CF was constant, the erosion geometry and higher QR resulted in higher stress concentration; but, when QR was kept constant, the higher CF in the abfraction cavity showed lower MPS concentration. These results are consistent with Rodrigues and others,[14] who rejected the hypothesis that interfacial shrinkage stresses between adjoining walls in cavities increases with CF, and with other authors[31-35] who have said that using CF as a single predictor for shrinkage stress has not been universally accepted.

When the margin is placed completely within enamel of bonded restorations, performance is thought to be more predictable, but frequently, Class V lesions extend onto the root surface and poor gingival margin adhesion can increase microleakage. In this study, Figures 3 and 4 show that the angle of the margin combined with the E modulus of the dental tissue influenced polymerization stress distribution. This suggests that beveling the gingival margin that ends in cementum should decrease MPS and VMS and, so, may decrease the microleakage in this region. This is in opposition to the data published by Owens and others,[36] who observed that nonretentive restorations, without a gingival bevel, exhibited significantly less microleakage along the gingival wall and less overall microleakage than did the beveled restorations. But, with *in vitro* testing, other factors, such as light exposure[37] or dentin humidity, that may affect results can be easily modified by the operator.

The effect of geometry on the gingival microleakage has not been tested, while nonsignificant differences were found when comparing the influence of different composites having different mechanical properties. These authors observed a modest decrease in microleakage associated with shrinkage stress for the same cavity shape.[38,39]

In the different clinical situations studied, abfraction and erosion cavities are associated with specific interactions with the tissues involved. This study supports moving the stress concentration away from the margin by increasing the angle of the cavosurface bevel because the stress at the enamel margin can decrease the lifetime of a restoration.

In Figure 3, the second column shows the effect of CF on the three different cavities. The lower the CF,

the higher the calculated stress. This was consistent with the observation of El-Sahn and others,[40] who showed that increasing the CF did not negatively affect the bond strength of low-shrinkage composites.

Comparing lesions of beveled abfraction geometry, even with an increase in QR and the resulting increase in CF, the peak stress decreased. This can be explained by a considerable difference in the geometry of this type of cavity: the pulpal wall became thinner (due to higher amounts of resin) and, therefore, more flexible. This may have contributed to the stress decrease during the resin polymerization. This also means that, in the abfraction lesion, the change in geometry overcame the expected effects of increasing CF and amount of resin. This finding about geometry is in accordance with Braga and others.[9] They concluded that shrinkage stress and microleakage in cylindrical restorations are influenced by both their diameter and depth, although cavity depth was found to have a stronger influence than diameter.

Considering the general values of stress (and not just the values achieved at the cavosurface angles), the case that seems more favorable for bond integrity is the abfraction beveled cavity with an area equal to 2.79 mm^2 because it presented, in a general manner, lower stress values.

According to the results obtained, the null hypothesis was rejected because the QR, the design of the cavity, and the bevel influenced the polymerization shrinkage stress. The findings indicate that the cavity geometry is the most important factor to be considered along with the presence or absence of a bevel. Appropriate application of this information can help to decrease the stress generated during resin composite polymerization. In addition, these findings mean that the stress varies among different types of cavities and that results measuring marginal integrity from laboratory tests should only be compared when walls of similar geometry are used.

CONCLUSIONS

Within the limitations of this methodology, it is possible to conclude that C-factor, quantity of restorative material, cavity geometry, and the angle formed at the cavosurface margin, as well as their combined interaction, influence stress distribution in different ways. The occlusal enamel cavosurface margin angle was the most relevant factor in predicting the stress in a Class V cavity.

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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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Inactivation of Matrix-bound Matrix Metalloproteinases by Cross-linking Agents in Acid-etched Dentin

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Clinical Relevance

Cross-linking agents used in clinically applicable periods of time are capable of inactivating matrix-bound matrix metalloproteinases (MMP) in demineralized dentin. Such treatment may render the hybrid layer less prone to degradation over time and produce long-lasting resin-dentin bonds.

SUMMARY

Objectives: Published transmission electron microscopy analysis of *in vitro* resin-dentin bonds shows that, after 44 months, almost 70% of collagen fibrils from the hybrid layer disappear. Matrix metalloproteinases (MMPs) play an important role in that process and are thought to be the main factor responsible for the solubilization of dentin collagen. Therefore, this study aimed to evaluate the inactivation of matrix-bound MMPs by two different cross-linking agents, carbodiimide (EDC) or proanthocyanidin (PA), or the MMP-inhibitor, chlorhexidine (CHX), on acid-etched dentin using a simplified MMP assay method.

Materials and Methods: Dentin beams (2×1×6 mm) were obtained from mid-coronal dentin of sound third molars and randomly divided into six groups (G) according to the dentin treatment: G1: Deionized water (control); G2: 0.1 M EDC; G3: 0.5 M EDC; G4: 0.5 M EDC + 35% hydroxyethyl methacrylate (HEMA); G5: 5% PA; and G6: 2% CHX. The beams were etched for 15 seconds with 37% phosphoric acid, rinsed, and then immersed for 60 seconds in one of the treatment solutions. The data were expressed both in absorbance values at 412 nm and in MMP-9 activity equivalents. The total MMP activity of dentin was analyzed for one

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hour by colorimetric assay (Sensolyte). Data were submitted to Wilcoxon nonparametric test and Mann-Whitney tests ($p>0.05$).

Results: All experimental cross-linking solutions significantly reduced MMP activity from 79.8% to 95.2% when compared to the control group. No difference was observed among 0.1 M EDC (84.8%), 5% PA (87.6%), and 2% CHX (79.8%). Addition of 35% HEMA to 0.5 M EDC produced inactivation (95.2%) that was similar to that of 0.5 M EDC alone (92.7%).

Conclusion: Dentin treatment with cross-linking agents is effective to significantly reduce MMP activity. Mixing 0.5 M EDC and 35% HEMA did not influence EDC inhibitor potential.

INTRODUCTION

Since the introduction of the total-etching concept by Fusayama[1] in 1980, the effects of acid-etching of dentin have been subject to many studies. Etching dentin with 32-37% phosphoric acid removes the mineral content of the top 10 μm of dentin and exposes the collagen fibrils of the matrix, thereby creating space for monomer infiltration to achieve micromechanical retention of adhesive resins.[2] Although acid-etching of dentin provides satisfactory initial bond strength, those bond strengths fall over time, raising concerns about the long-term stability of adhesive-resin restorations.[3]

Resin/dentin bond degradation is a complex process that is not completely understood, involving the hydrolysis of both the resin and the collagen component of the hybrid layers. Acid-etched dentin contains bound matrix metalloproteinases (MMPs)-2, -3, -8, -9, and -20 and cathepsins[4,5] in their active forms. These enzymes are exposed and activated by acid-etching and can slowly degrade collagen fibrils[6-9] within the hybrid layer, resulting in a significant bond strength loss of 36-70% between 12 and 14 months.[10,11]

In order to reduce the activity of these proteases and preserve the long-term integrity of adhesive interfaces, chlorhexidine (CHX) has been used as a nonspecific inhibitor of MMPs.[6-9,11-13] CHX is also an effective inhibitor of cysteine cathepsins.[14] However, this substance is water-soluble and may undergo leaching from the hybrid layer, which impairs its long-term anti-MMP effectiveness.[13]

A new alternative to the inhibition of proteases by inhibitors is the treatment of demineralized dentin with cross-linking agents that can inactivate the catalytic site of these enzymes.[15] The cross-linker 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) is capable of forming covalent peptide bonds between proteins by activating the free carboxyl groups of glutamic and aspartic acids present in protein molecules.[16,17] This results in the formation of a o-acylisourea intermediate that reacts with the epsilon amino group of lysine or hydroxylysine in an adjacent polypeptide chain to form a stable, covalent amide bond. The only by-product of the reaction is urea,[18,19] which is water-soluble and easily removed from dentin by water rinsing. Furthermore, 0.5 M EDC shows no transdentinal cytotoxicity on odontoblast-like cells (Scheffel and others, unpublished data) and is able to increase the mechanical properties of the collagen matrix.[20]

Other cross-linking agents, such as the proanthocyanidins (PAs), are polyphenolic natural products composed of flavan-3-ol subunits linked mainly through C4-C8 (or -C6) bonds.[21] This substance is widely present in fruits, vegetables, nuts, seeds, flowers, and barks and shows numerous biological activities, such as antioxidant capacity,[22] antimicrobial effects,[23] anti-inflammatory properties,[24] positive effects on cardiovascular diseases,[25] and antiallergic activity.[26]

Thus, the purpose of this study was to evaluate the inactivation of matrix-bound MMPs by topical application of cross-linking agents on acid-etched dentin. The null hypothesis was that cross-linker–treated and –untreated dentin do not differ regarding MMP activity.

MATERIALS AND METHODS

Thirty extracted human third molars were obtained from 18-21-year-old patients with informed consent under a protocol approved by the Georgia Regents University. The teeth were stored frozen until required. After thawing, the enamel and superficial dentin were removed using an Isomet saw (Buehler Ltd, Lake Bluff, IL, USA) under water cooling. One 1-mm-thick dentin disk was produced from the midcoronal dentin of each tooth. Then 60 dentin beams (2×1×6 mm) were sectioned from the dentin disks. One such beam was placed in each well. This represents 40 mm^2 of dentin, which is equivalent to a Class I cavity prepared in a mandibular first molar 2 mm into the dentin and 3×4 mm in dimension. The beams were etched by dipping them into 37% phosphoric acid (pH, −0.5) for 15 seconds and then copiously rinsing with deionized water for 15 seconds. The beams were randomly divided into six groups (n=10) according to the dentin treatment, as

follows: G1: Deionized water (positive control) (pH 6.73); G2: 0.1 M EDC (pH 6.07); G3: 0.5 M EDC (pH 6.24); G4: 35 vol% hydroxyethyl methacrylate (HEMA) in water + 0.5 M EDC (pH 6.34); G5: 5% PA (Polyphenolics Inc, Madera, CA, USA) (pH 5.2) in phosphate-buffered saline (pH 6.0); and G6: 2 vol% CHX digluconate (negative control) in water (pH 6.43). All beams were dipped in the treatment solutions for 60 seconds and rinsed with distilled water for 10 seconds, except for 2% CHX, in which case the beams were only blot dried. After the treatment, each beam was placed in a 200 μL/well containing generic MMP substrate (Sensolyte Generic MMP colorimetric assay kit; catalog No. 72095, AnaSpec Inc, Fremont, CA, USA) for 60 minutes at 25°C in a 96-well plate. At the end of 60 minutes, the total MMP activity was determined by measuring the absorbance of the wells at 412 nm in a plate reader (Synergy HT microplate reader, BioTek Instruments, Winooski, VT, USA) against appropriate blanks. All chemicals were purchased from Sigma/Aldrich Chemical Co. The generic MMP assay uses a proprietary thiopeptide to assay MMP-1, -2, -3, -7, -8, -9, -12, -13, and -14. Thus, the kit measured the total endogenous MMP activity of dentin, with the exception of MMP-20 (enamelysin). A standard curve of absorbance of the substrate vs rh MMP-9 activity (ng) was constructed to permit expression of total MMP activity in MMP-9 equivalents. The rh MMP-9 was activated using trypsin at a final concentration of 10 μg/mL, pH 7.4, at 37°C for two hours. Then the trypsin was inactivated by addition of trypsin inhibitor at a final concentration of 100 μg/mL. Human recombinant MMP-9 was purchased from Calbiochem (catalog No. PF038; Billerica, MA, USA). Its specific activity was 1300 pmoles/mg.

Statistical Analysis

For determination of MMP activity, the absorbance data set was submitted to Wilcoxon nonparametric test and Mann-Whitney test at the 5% level of significance. The percentage of MMP activity inhibition was calculated based on the water control group MMP activity and MMP-9 equivalents (ng/well) based on the rh MMP-9 curve.

RESULTS

When mineralized dentin beams were dipped in 37 wt% phosphoric acid for 15 seconds and then rinsed with water, the top 8-10 μm of the beams were completely demineralized (Figure 1). When etched dentin beams were dipped in water (control) and then dropped into the generic MMP substrate, the absorbance at 412 nm gradually increased to 0.51 (±0.138) over 60 minutes. That value was considered to represent 100% of the total MMP activity in the etched dentin, and it was used to calculate the

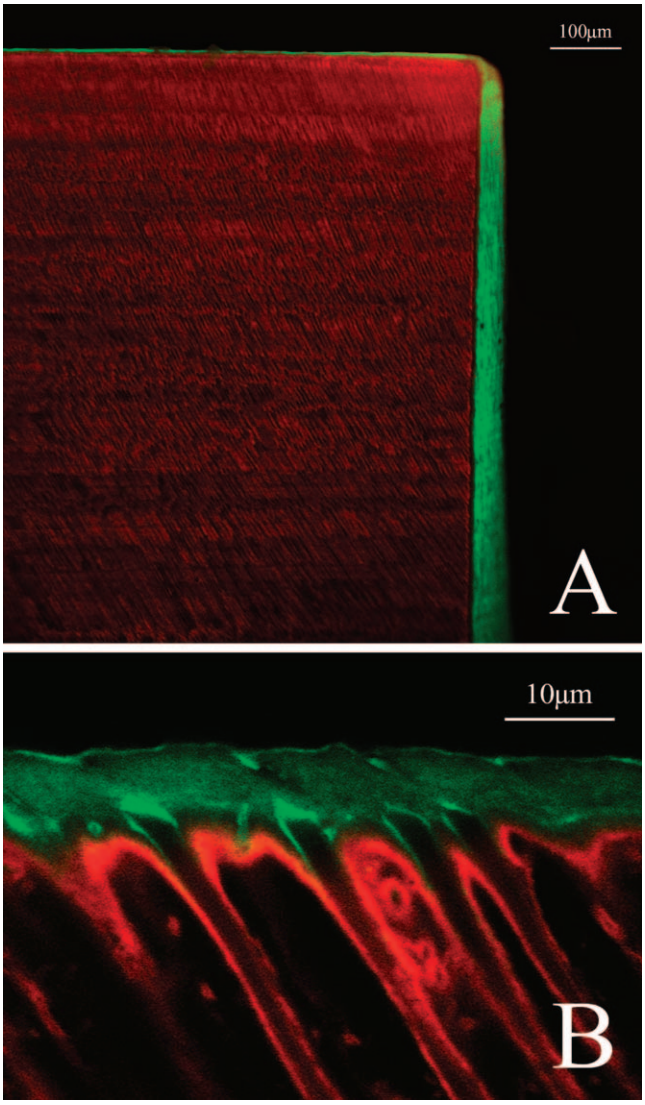

Figure 1. *Confocal laser scanning microscope (CLSM) images of the etched layer shown dyed green on the surface of a dentin beam. Dentin beams were etched in 37% phosphoric acid for 15 seconds and then rinsed with deionized water for 60 seconds and labeled for five hours, respectively, with 1% w/v fluorescein isothiocyanate (FITC) in anhydrous dimethyl sulfoxide (DMSO) and 1% w/v xylenol orange (XO) in water. The two fluorochromes selectively label collagen (FITC) and the mineralized matrix (XO), respectively. Prior to CLSM observation, the slabs were rapidly blotted with absorbent paper to remove the excess of fluorochrome, mounted on glass slides, and promptly examined. Samples were scanned in two-channel fluorescence mode with both 488 nm excitation–525 nm emission (green channel) and 546 nm excitation–580 nm emission (red channel), respectively, for FITC and XO labeling. (A) 10× projection of 53 images (final Z-stack thickness: 346 μm); the sample was intentionally tilted to highlight the peripheral distribution of demineralized collagen. (B) 100× image of the border between demineralized surface collagen fibrils (etched layer) and underlying mineralized dentin matrix.*

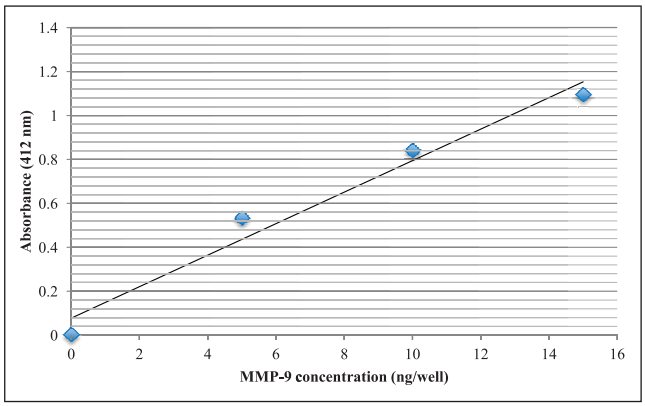

Figure 2. *Standard curve of rh matrix metalloproteinase (MMP)-9 activity (ng/well) vs absorbance at 412 nm after 60 minutes.*

percentage of MMP activity inhibition of the investigated cross-linking agents and CHX. A standard curve of substrate absorbance at 60 minutes vs ng of rh MMP-9 is shown in Figure 2. All cross-linking agents significantly reduced MMP activity in acid-etched dentin after 60 seconds of topical treatment (Table 1). The percentage of MMP inhibition for the EDC solutions, PA, and CHX ranged from 79.8% for 2 wt% CHX to 95.2% for 0.5 M EDC + 35% HEMA (Table 1). There was no statistical difference in MMP activity when 0.1 M EDC, 5% PA, and 2% CHX were compared (Table 1). When 0.5 M EDC was mixed with 35% HEMA to simulate the composition of an adhesive primer, the HEMA did not interfere with that cross-linker in inactivating the total MMP activity of acid-etched dentin. When the absorbances of the MMP activity of acid-etched dentin were expressed in MMP-9 activity equivalents, the total MMP activity of acid-etched dentin was equivalent to 6.10 (±1.93) ng of MMP-9 per 2 × 1 × 6 mm of acid-etched dentin.

DISCUSSION

The conventional method for analyzing the total bound MMP activity using Sensolyte Generic MMP colorimetric assay kit includes the complete demineralization of dentin beams for 18 hours with 10% phosphoric acid.[27] The current study used a simplified MMP assay method in which the dentin was acid-etched for 15 seconds with 37% phosphoric acid. That avoids the complete dentin demineralization and reproduces more closely the surface demineralization of dentin that is completed during etch-and-rinse bonding procedures. The complete demineralization of the dentin beam creates a much deeper collagen area (2×1×6 mm) to be infiltrated by the cross-linking solutions and adhesive resins. Clinically, acid-etching of dentin by 37 wt% phosphoric acid for 15 seconds only demineralizes dentin to a depth of 8-10 μm (Figure 1). Such relatively thin zones of demineralized dentin are easily saturated by test solutions within seconds. Nevertheless, this technique does not reproduce all *in vivo* conditions, such as the presence of pulpal pressure and the outflow of dentinal fluid.

The hybrid layer is composed of 30 vol% collagen[28] (primarily type I), while the other 70% corresponds to resin and residual solvent.[2] The collagen fibril network acts as an anchorage to resin, enabling the retention of adhesive restorations. However, transmission electron microscopy analyses revealed that almost 70% of collagen from the adhesive interface disappears after 44 months of water storage.[29] Proteases such as metalloproteinases (MMPs) and cysteine cathepsins are thought to be responsible for collagen fibril enzymatic degradation via hydrolysis.[30]

Exogenous MMP inhibitors have been tested in order to reduce protease activity and prolong the durability of resin-dentin bonds. CHX was the first

Table 1: *Absorbance, Percent Inactivation/Inhibition of Total Matrix-bound Matrix Metalloproteinase (MMP) Activity in Dentin, and MMP-9 equivalent (ng/dentin beam)*[1]

Demineralized Dentin Treatment	Absorbance (412 nm)	MMP Inhibition, %	MMP-9 Equivalent, ng
Water (control)	0.515 (±0.138) A	0 D	6.10 (±1.93) A
0.5 M EDC	0.038 (±0.014) CD	92.7 (±2.6) AB	0 (±0.19) CD
0.5 M EDC + 35% HEMA	0.025 (±0.016) D	95.2 (±3.0) A	0 (±0.22) D
0.1 M EDC	0.078 (±0.042) BC	84.8 (±8.2) BC	0 (±0.59) BC
5% PA	0.064 (±0.035) BC	87.6 (±6.7) BC	0 (±0.49) BC
2% CHX	0.104 (±0.031) B	79.8 (±6.0) C	0.37 (±0.43) B

Abbreviations: CHX, 2 wt% chlorhexidine digluconate; EDC, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride; HEMA, 2-hydroxyethyl methacrylate; PA, grape seed extract containing proanthocyanidins.

[1] *Values are mean (±standard deviation) absorbance, % inhibition of total MMP activity of dentin measured by SensoLyte substrate (AnaSpec Inc, Fremont, CA, USA), and MMP-9-equivalent (ng of MMP-9/dentin beam). Within each column, groups identified by different letters are significantly different (Mann-Whitney, p<0.05).*

MMP inhibitor proposed for such a purpose during bonding to dentin.[31] It has been largely studied as a nonspecific MMP[12] and cathepsin inhibitor.[14] CHX adsorbs on dentin and decreases hybrid layer degradation *in vitro*[7,32-34] and *in vivo*.[6,8,11,13] However, this inhibitor is soluble in water and can slowly leach from the adhesive interface over time,[13] since no chemical bond is established between the CHX molecule and the collagen fibril.

One of the mechanisms proposed to explain how MMPs degrade collagen is that these proteases unwind collagen molecules when they bind to them. By doing that, the endogenous protease's active site is allowed sufficient space to attack the specific glycine-isoleucine peptide bond in peptide chains.[35-37] Cross-linking agents stiffen collagen polypeptides so that they cannot unwind, and they can also inactivate the catalytic site of proteases[38] by creating a new peptide bond across adjacent peptides. Hence, it is reasonable to expect that MMP inactivation by cross-linking agents should last much longer than the inhibition of proteases by matrix-bound CHX. EDC and PA were first used to increase the modulus of elasticity of collagen and make it more difficult for MMPs to unwind the collagen triple-helix structure. However, EDC and PA are still not capable of increasing the stiffness of collagen in clinically relevant periods of times, such as 30 seconds and 60 seconds (Scheffel and others, unpublished data).

Despite the long application times that cross-linking agents require to increase collagen stiffness,[20] they are effective against MMPs in 60 seconds. The results of this study require rejection of the tested null hypothesis. All investigated solutions significantly decreased MMP activity in acid-etched dentin within 60 seconds. Five percent PA, 0.1 M EDC, and 0.5 M EDC were all able to inactivate more than 84% of the total active MMPs. EDC activates the free carboxylic acid groups of glutamic and aspartic acids without introducing additional methylene groups. MMPs-2 (EC 3.4.24.24), -8 (EC 3.4.24.34), -9 (EC 3.4.24.35), and -20 (EC 3.4.24), the MMPs reported to be in dentin matrix, have glutamic acid in their active sites in positions 404, 218, 402, and 227, respectively, allowing EDC to react to those sites. Additionally, the concentrations of EDC tested in this study did not produce any evidence of transdentinal cytotoxic effect on odontoblast-like cells in separate experiments (Scheffel and others, unpublished data), where they were also used at 0.1 M and 0.5 M, making EDC safe for *in vivo* application. The use of 0.5 M EDC was designed to accelerate its rate of diffusion into demineralized dentin. That EDC concentration is far in excess of the amount of protein in demineralized dentin. It is likely that only 1-2% of the EDC could react with proteins in 60 seconds. This would only generate 0.005-0.01 M of urea, which is not sufficient to denature any proteins. Denaturing concentrations of urea require 2-8 M.[39,40]

PA is a natural plant cross-linking agent. The mechanism of cross-linking is not completely understood. There are four different theories to explain how PA interacts with proteins. They include covalent,[41] ionic,[42] hydrogen bonding,[43] and hydrophobic interactions.[44] This substance has been reported to increase the stiffness of demineralized dentin[45] and to inhibit the progression of artificial root caries.[46,47] Additionally, scanning electron microscopy of demineralized dentin collagen treated with 15% PA for periods shorter than 120 seconds showed a homogeneous and regular collagen fibril arrangement, regardless of the surface moisture condition.[38] That result indicates that, in addition to acting as MMP inhibitor cross-linking agents, it can stiffen demineralized dentin sufficiently to minimize the risk of collagen network collapse resulting from air-drying. However, the PA solution has a dark color, which stains the dentin despite water rinsing. That could be a drawback for the clinical use of this cross-linker. Its rapid, complete inactivation of matrix-bound MMPs in dentin indicates that more research should be done to try to isolate an uncolored fraction of the PA.

When 0.5 M EDC was solubilized in 35 vol% HEMA, there was no reduction in its ability to inactivate all of the MMPs in dentin. That is, it was as effective as 0.5 M EDC alone. Since HEMA is an important component of adhesives, it may be possible to mix EDC with HEMA and other primer components in etch-and-rinse adhesive systems to inactivate MMPs during bonding. However, it is not known whether EDC influences adhesive polymerization. Further studies are still needed to demonstrate the effects of short-time application of cross-linking agents over time *in vitro* and *in vivo*.

CONCLUSIONS

Dentin treatment with cross-linking agents is effective in significantly reducing MMP activity; 0.5 M EDC showed the best results. Mixing 0.5 M EDC and 35% HEMA did not influence EDC cross-linking of MMPs, indicating that EDC could be added to primers in adhesive systems.

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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 Pre-reacted Glass-ionomer Filler Extraction Solution on Demineralization of Bovine Enamel

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Clinical Relevance

S-PRG filler extraction solution has an ability to protect the surface of enamel from demineralization.

SUMMARY

Objective: To determine the effect of pre-reacted glass-ionomer (PRG) filler extraction solution on the demineralization of bovine enamel by measuring changes in the ultrasound transmission velocity.

Methods: The specimens were prepared by cutting bovine teeth into enamel blocks. The specimens were immersed in buffered lactic acid solution for 10 minutes twice a day, and then stored in artificial saliva. Other specimens were stored in PRG filler extraction solution for 10 minutes, followed by 10-minute immersion in the buffered lactic acid solution twice a day. The propagation time of longitudinal ultrasonic waves was measured by a pulser receiver. Six specimens were used for each condition, and analyses of variance followed by Tukey tests (α=0.05) were done.

Results: No changes in sonic velocity were found for specimens stored in the PRG filler extraction solution, indicating that the PRG extraction solution had an effect on inhibiting the demineralization of bovine enamel.

Conclusions: The results obtained with the use of an ultrasound measurement technique suggested that PRG filler extraction solution has the ability to prevent demineralization of enamel.

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INTRODUCTION

The development of erosion involves a chemical process in which the inorganic phase of the tooth is demineralized, thereby reducing the hardness of the

tooth substrates.[1] Subsequent abrasive challenges through brushing increase the loss of the tooth substrates.[2] Dietary changes and inadequate oral hygiene have led to erosion becoming more frequent among young people. This phenomenon is largely due to physical and chemical factors that act in the area of the tooth neck, resulting in enamel loss and dentin exposure. Even in populations with a decreased prevalence of caries, the relative importance of occlusal wear has significantly increased. The inclusion of active ingredients in oral-care products to help prevent enamel loss may contribute greatly to the improvement and maintenance of oral health.

Pre-reacted glass-ionomer (PRG) filler is prepared by an acid-base (glass ionomer) reaction between fluoroaluminosilicate glass and polyacrylic acid in the presence of water, preliminarily forming a stable glass-ionomer phase within the glass particles.[3,4] Upon freeze-drying, the desiccated xerogel is further milled and silane-treated to form PRG fillers of a specific size range. Full reaction-type and surface reaction-type (S-PRG) PRG fillers can be prepared, and this technology is used in the formulation of "giomer" products.[5]

Both types of PRG fillers promote rapid fluoride release through ligand exchange within the pre-reacted hydrogel.[6] The gel phase of the glass core acts as a source of released ions, as the reaction between polyacid and glass powders is thought to produce soluble ions, many of which are released from PRG fillers into the surrounding solution. A previous study showed that PRG filler extraction solution had a modulation effect on an acidic environment as a result of ion release.[7] The release of considerable levels of aluminum (Al), boron (B), fluorine (F), sodium (Na), silicon (Si), and strontium (Sr) from S-PRG filler into surrounding distilled water was detected.[7] Furthermore, although minor amounts of Na are present in S-PRG filler compared with F and Si, more Na was released than other ions. As Na is the only cation eluted to preserve electroneutrality, an equivalent amount of cations is expected to be released.[8] During previous acidic attachment to the glass powder, F and Na were liberated into the matrix, and together formed soluble salt.[9] This might contribute to a rapid release of F into the PRG filler extraction solution, preventing demineralization of the enamel substrate.

Ultrasonic imaging is a noninvasive technique that shows considerable diagnostic potential as well as being a valuable research tool.[10,11] Ultrasonic devices can be used to detect carious lesions[12] and to measure the dentin thickness between the tooth surface and the pulp chamber.[13] Assuming that the enamel substrate is mainly composed of hydroxyapatite, differences in ultrasonic velocity can be related to differences in the degree of mineralization and histologic structures, as the ultrasonic velocity increases proportionally with the volumetric concentration of minerals.[14] Ultrasonic velocity has also been shown to be related to the mineral content of the enamel lesion body, and so is an index of the degree of mineralization.[15] When the tooth substrate suffers demineralization, the mineral volume concentration at the tooth surface, as well as the specific ultrasonic velocity, decreases.

The present study evaluated the effect of PRG filler extraction solution on enamel demineralization by the measurement of changes in ultrasonic velocity using an ultrasonic device, and by observation using scanning electron microscopy (SEM). The null hypothesis was that PRG ion leaching prevented demineralization of enamel substrate.

MATERIALS AND METHODS

Fluoroboroaluminosilicate glass was prepared by fusing 14.0 wt% silica (SiO_2), 27.0 wt% mullite ($3Al_2O_3 \cdot 2SiO_2$), 19.0 wt% boric oxide (B_2O_3), 5.0 wt% cryolite (Na_3AlF_6), 29.0 wt% strontium fluoride (SrF_2), and 6.0 wt% strontium carbonate ($SrCO_3$). The mixture was heated and melted in an arc furnace at 1400°C for two hours. The melted mixture in liquid form was removed from the furnace and quenched in running water, then dried for 12 hours at 150°C in an air oven to obtain glass frit. Analysis of the glass frit with an X-ray fluorescence spectrometer (ZSX100e, Rigaku Corp, Tokyo, Japan) and an X-ray diffractometer (Multiflex 2 kW, Rigaku Corp) showed that the structure was amorphous and consisted of 21.6 wt% SiO_2, 21.6 wt% Al_2O_3, 16.6 wt% B_2O_3, 27.2 wt% SrO, 2.6 wt% Na_2O, and 10.4 wt% F.

The glass frit was coarsely ground with a ball mill (BM-10, Seiwa Giken Co, Hiroshima, Japan) and then wet-ground with an agitator bead mill in the presence of water to obtain irregular-shaped filler particles. The resulting glass slurry was agitated with the addition of polysiloxane (Mitsubishi Chemical Co, Tokyo, Japan) solution (SiO_2 content, 16 wt%), then aged at 50°C for 40 hours, and heat-treated at 120°C for six hours in a heat dryer. The heat-treated solidified material was then disintegrated in a high-speed mixer (FS-GC-20JE, Fukae Powtec Co, Osaka, Japan) to obtain surface-treated glass filler. During stirring in the cutter mixer, the glass filler was subjected to spray treatment with

polyacrylic acid aqueous solution (polymer content, 13.0 wt%) and then treated in a heat dryer at 150°C for three hours to obtain S-PRG filler. The mean particle size of the S-PRG filler was 3.0 μm, as measured using a laser-diffraction particle-size analyzer (Microtrac HRA 9320-X100, Nikkiso Co, Tokyo, Japan).

Distilled water (pH 5.9) was mixed with S-PRG filler at a 1:1 ratio (1 L:1000 g) by weight. The mixture was stirred for 24 hours and centrifuged to precipitate S-PRG filler. The supernatant solution was filtrated using a chromato disk (25A hydrophilic type, diameter 25 mm, pore size 0.2 μm, GL Sciences Inc, Tokyo, Japan) to obtain the test liquid. Elemental analysis of ions (Al, B, Na, Si, and Sr) released from S-PRG filler was performed using inductively coupled plasma atomic emission spectroscopy (ICPS-8000, Shimadzu Co, Kyoto, Japan). Analysis was conducted after preparing calibration curves corresponding to each element. Concentration of F was also analyzed using a fluoride electrode (9609BN, Orion Research Inc, Jacksonville, FL, USA) connected to a pH/ion meter (720A, Orion Research Inc) after preparing calibration curves. The amount (mg/g) of ions released from S-PRG filler was 0.04 for Al, 2.07 for B, 0.09 for F, 0.51 for Na, 0.03 for Si, and 0.25 for Sr.[7]

In total, 18 freshly extracted bovine incisors, without cracks or erosion, were cleaned and stored in physiologic saline for up to two weeks. The teeth were sliced longitudinally at a 1-mm thickness and then cut in the buccolingual direction with a low-speed diamond saw (Buehler Ltd, Lake Bluff, IL, USA). Each slab was carefully shaped into a rectangular form ($4 \times 4 \times 1$ mm) using a super-fine diamond finishing point (ISO #021, Shofu Inc, Kyoto, Japan). Specimen surfaces were ground successively on wet silicon carbide paper with a grit size of 600, 1200, and 2000. The thickness and size of the specimens were measured using a dial gauge micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan).

The specimens in the demineralization group (n=6) were treated with undersaturated 0.1 M lactic acid buffer solution (pH 4.75, 0.75 mM $CaCl_2 \cdot 2H_2O$, and 0.45 mM KH_2PO_4) for 10 minutes and then placed in artificial saliva (pH 7.0, 14.4 mM NaCl, 16.1 mM KCl, 0.3 mM $MgCl_2 \cdot 6H_2O$, 2.0 mM K_2HPO_4, 1.0 mM $CaCl_2 \cdot 2H_2O$, and 0.10 g/100 mL sodium carboxymethyl cellulose). These procedures were performed twice daily (interval time 10 hours) over the four-week test period, and the specimens were stored between treatments in artificial saliva at 37°C. The specimens in the PRG group (n=6) were stored in PRG extraction solution for 10 minutes prior to storage in demineralizing undersaturated 0.1 M lactic acid buffer solution. The specimens in the control group (n=6) were stored in artificial saliva for the same period of time.

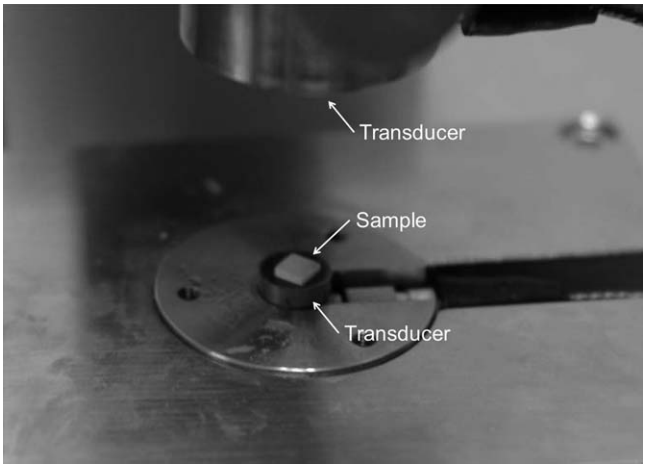

Figure 1. *Image of testing setup.*

The ultrasonic velocity was measured using a pulser receiver (Model 5900PR, Panametrics, Waltham, MA, USA), a transducer for longitudinal waves (V112, Panametrics), and an oscilloscope (Wave Runner LT584, LeCroy Corp, Chestnut Ridge, NY, USA).[16] Measurements were taken before the test, and then on days 1-7, 14, 21, and 28. The equipment was initially calibrated using a standard procedure with 304 stainless steel calibration blocks (2211M, Panametrics) with thicknesses of 2.5, 5.0, 7.5, 10.0, and 12.5 mm.

The transducer was oriented perpendicularly to the contact surface of each specimen, to obtain the echo signal (Figure 1). The ultrasonic waves propagated from the transducer to the tooth were transmitted through the tooth and were detected by the transmitter on the opposite side. Each measurement was conducted at 23 ± 1°C and 50 ± 5% relative humidity.

The ultrastructural observation of enamel surfaces was carried out using field-emission (FE) SEM. Specimens were dehydrated in ascending concentrations of tert-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes, and 100% for two hours) and then transferred to a critical-point dryer for 30 minutes. The surfaces were coated in a vacuum evaporator (Quick Coater Type SC-701, Sanyu Denshi Inc, Tokyo, Japan) with a thin film of Au. Specimens were observed by FE-SEM (ERA

Table 1: *Average Ultrasonic Velocities (m/s) of Bovine Enamel Specimens by Treatment*[a]

Group	Treatment Time, d				
	0	7	14	21	28
Control	6110 (80)	6114 (99)	6112 (101)	6113 (105)	6114 (108)
De	6050 (165)	5663 (140)[bc]	5598 (143)[bc]	5580 (146)[bc]	5523 (150)[c]
PRG	6129 (80)	6251 (98)	6267 (92)	6281 (94)	6290 (94)

Abbreviations: De, demineralization group; PRG, prereacted glass-ionomer filler extraction solution.
[a] *Data are shown as mean (standard deviation). n=6 per group.*
[b] *Significant within-group difference from mean at days 0 and 28.*
[c] *Significant between-group difference.*

8800FE, Elionix Ltd, Tokyo, Japan) at an accelerating voltage of 10 kV.

The ultrasonic velocity data were analyzed by two-way analysis of variance (ANOVA), with time and treatment as factors; time was treated as a repeated measure. *Post hoc* pairwise tests among groups were performed using the Tukey test. The level of significance (p-value) was 0.05. Calculations were performed using Sigma Stat software version 3.1 (SPSS Inc, Chicago, IL, USA).

RESULTS

The average ultrasonic velocities of the enamel specimens are shown in Table 1 and Figure 2. The differences between storage periods were greater than expected by chance after allowing for the effects of storage conditions, so multiple comparisons were conducted on the data. The average ultrasonic velocity in intact bovine enamel (control group) ranged from 6090 to 6119 m/s and did not vary significantly with treatment time. The ultrasonic velocities in the demineralization group decreased and were significantly lower than those in the control group after seven days. There was no significant change in the ultrasonic velocity with treatment time in the PRG group, and no significant difference from the control group was detected up to 28 days.

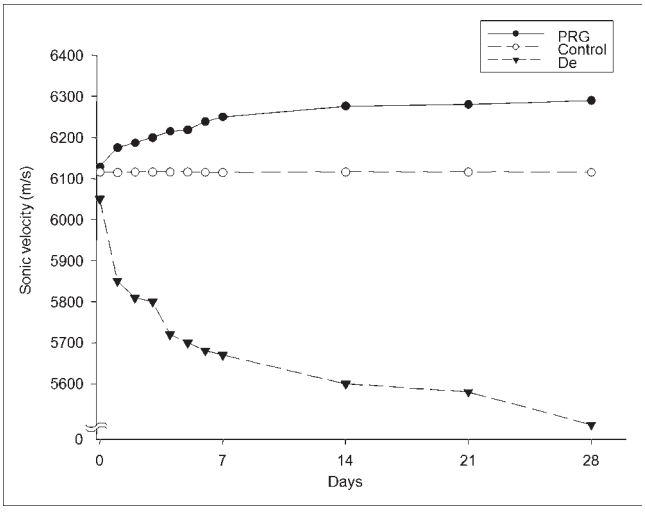

Figure 2. *Influence of storage conditions on changes in ultrasonic velocities of enamel specimens.*

Representative SEM images of enamel specimens are shown in Figure 3. SEM images of the enamel specimens revealed morphologic differences in treatment effects. Pronounced demineralization of enamel surfaces was observed over the test period in the demineralization group, whereas the PRG group showed relatively minor or no morphologic change.

DISCUSSION

Comparative data on the properties of human and bovine hard dental tissue are scarce; however, bovine enamel is widely used as a substitute for human enamel.[17] Human teeth are thought to be most relevant for conducting *in vitro* studies.[18] However, bovine teeth were used in the present study because they are easy to obtain in large quantities, are in good condition, and have fewer composition variables. Bovine teeth have large flat surfaces and have not undergone prior caries challenges that might affect test results.[19] Moreover, structural changes and the mineral distribution of

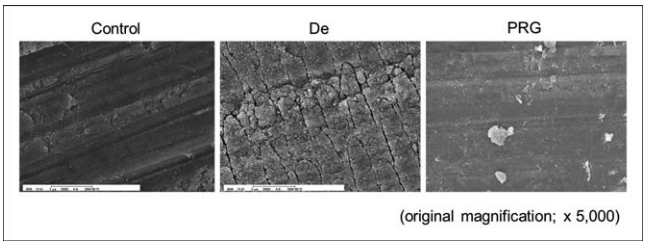

Figure 3. *Representative FE-SEM images of dentin surfaces (scale bar, 5 μm). SEM observations revealed differences in morphologic features among storage conditions. Demineralization of the enamel surfaces was more pronounced in the demineralization group, whereas the PRG group showed only slight morphologic changes compared to those of the control group.*

carious lesions are reported to be similar in human and bovine teeth.[20]

Enamel is a mineralized material with a highly complex hierarchical structure that is composed mainly of aligned rods arranged almost perpendicular to the tooth surface. Enamel should be modeled as an anisotropic material,[21] with both the longitudinal section and the buccal surface shown to be elastically anisotropic, so the orientation of the enamel prism might modify the ultrasonic velocity.[22] In a longitudinal section, the anisotropy appears to be closely related to the prismatic orientation.[23] The enamel prisms are at different angles to the direction of the ultrasonic beam according to the tooth section plane. Therefore, to avoid any effect on ultrasonic velocity, we obtained enamel specimens from the labial surfaces of bovine teeth.

Our results demonstrated changes in the ultrasonic velocity of enamel in the control group as a function of demineralization time, although they were not linear (Figure 2). The ultrasonic velocities of specimens in the PRG group did not change significantly with time. Twice-daily application of PRG filler extraction solution resulted in the maintenance of normal ultrasonic velocity for the enamel, contrary to the reduced velocity observed in the demineralization group. These results are consistent with our previous study using casein phosphopeptide-amorphous calcium phosphate to prevent demineralization.[24] The PRG filler extraction solution therefore appeared to prevent enamel demineralization. These results were supported by the absence of signs of demineralization in the PRG group according to SEM (Figure 3). The S-PRG fillers released Al, B, F, Na, Si, and Sr ions.[7] Silicate and fluoride are known as strong inducers of remineralization of the dentin matrix.[25] Strontium and fluoride also improve the acid resistance of teeth by acting on hydroxyapatite to convert it to strontium apatite and fluoroapatite, respectively.[26]

Although the role of F in forming acid-resistant tooth substrate is well documented,[27] the influence of other ions is less well defined. Among the ions released from S-PRG filler, Sr is thought to play a role in tooth mineralization.[28] The effect of Sr on enamel remineralization was previously investigated, and it appeared to have the capacity to enhance enamel remineralization in conjunction with F.[29] Si is thought to promote hydroxyapatite formation, as hydroxyapatite nucleation has been shown to be triggered in the presence of silica gel.[30] Hydrated silica gel has sufficient silanol groups to induce apatite nucleation on its own surface; nucleation then proceeds by taking Ca and P from the surrounding environment. Another report suggested that Si released from bioactive glass particles is absorbed onto the substance, thus providing sites for heterogeneous CaP nucleation. Once nucleated, it spontaneously grows in solution to form a bone-like apatite layer.[31] The effects observed in the present study are likely to provide clinical benefits after long-term usage in the oral environment.

Oral hygiene products, such as toothpaste with antiplaque chemicals or functionally designed toothbrushes, have been shown to provide benefits in terms of plaque removal.[32] However, the consequences of abrasion from both toothbrushes and toothpaste are not fully understood.[33] Here we tested a liquid formulation of PRG filler extraction solution, which can be used similar to mouth rinse or applied with cotton swabs. The use of this solution might reduce sensitivity caused by home whitening procedures through the prevention of demineralization. The inclusion of active ingredients in oral-care products to help prevent dental disease has been shown to contribute greatly to the improvement and maintenance of oral health. Further research is needed into whether PRG-filler extract solutions can act as an oral hygiene material in clinical situations.

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that the S-PRG filler extraction solution has an ability to prevent demineralization of the enamel. Released ions such as Al, B, F, Na, Si, and Sr from the S-PRG fillers might improve the acid resistance of teeth.

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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 that is presented in this article.

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Transenamel and Transdentinal Penetration of Hydrogen Peroxide Applied to Cracked or Microabrasioned Enamel

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MO Gallinari • PH dos Santos

Clinical Relevance

Microabrasion and the presence of cracks in enamel make this substrate more susceptible to penetration of hydrogen peroxide during in-office whitening.

SUMMARY

The present study evaluated transenamel and transdentinal penetration of hydrogen peroxide during tooth whitening recognized in altered enamel by the presence of cracks or microabrasion. We used 72 experimental units (n=20) obtained from bovine incisors: GI-sound enamel; GII-teeth showing visible enamel cracks (4 mm to 5.7 mm in length); and GIII-microabrasioned enamel. The 12 remaining specimens were used to analyze the enamel surface morphology using scanning electron microscopy. The specimens were cylindrical and 5.7 mm in diameter and 3.5 mm thick. A product based on 35% hydrogen peroxide was used for bleaching, following the manufacturer's recommendations for use. To quantify the H_2O_2 penetration, the specimens were placed in artificial pulp chambers containing an acetate buffer solution. After bleaching, the solution was collected and adequately proportioned with leucocrystal violet, peroxidase enzyme, and deionized water. The resulting solution was evaluated using ultraviolet visible reflectance spectrophotometer equipment. The data were analyzed by analysis of variance (ANOVA) and Fisher's PLSD at a significance level of 0.05, and significant differences in the penetration

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of peroxide in different substrate conditions were observed ($p<0.0001$). The penetration of hydrogen peroxide was more intense in cracked teeth. The group in which the enamel was microabraded showed intermediate values when compared to the control group. Microabrasion and the presence of cracks in the enamel make this substrate more susceptible to penetration of hydrogen peroxide during in-office whitening.

INTRODUCTION

Although tooth bleaching has been considered a conservative technique[1-3] associated with ease of implementation and highly efficient performance,[4] there are concerns about the biologic safety of the procedure, referring to the indiscriminate use of highly concentrated peroxide products. Studies have shown that the diffusion of hydrogen peroxide through dental tissues may cause pulpal damage[5-7] and increased postoperative sensitivity.[8,9]

Even though the success of a bleaching treatment is directly related to the diffusion capacity of peroxides through enamel and dentin, it is believed that the more intense the penetration, the higher the risk of occurrence of side effects. Thus, bleaching is contraindicated in clinical conditions in which the tooth presents exposed dentin or other enamel tissue changes that presumably increase its permeability.

Cracks in the enamel are common but often are ignored or not noticed. These changes appear as fissures within the structure of the enamel and extend preferably along the cervico-incisal axis and may even reach the dentino-pulpal complex, which can also cause fractures in the tooth structure.[10-14] Thus, it seems possible that the presence of these changes may affect the intensity of hydrogen peroxide diffusion toward the pulp chamber.

Another common clinical condition refers to chromatic or textural changes on the enamel surface layers. In these cases, clinicians use a microabrasive treatment, which is an excellent choice for removing these changes by providing improved esthetics with a minimally invasive treatment.[15-19] Despite reports that the wear of microabrasion is small,[16,18,19] it is important to consider that the removal of the aprismatic layer and the conditioning provided by acids in these products can potentially alter the permeability of dental tissues.

Even though these clinical findings are relatively common, the literature shows no studies that quantify or compare the penetration levels of hydrogen peroxide when the bleach is applied in these conditions. Therefore, considering that the presence of cracks and enamel microabrasion represent tissue changes with potential influence on its permeability, studies are needed to assess the real impact of these changes on the intensity of hydrogen peroxide diffusion toward the pulp chamber.

MATERIALS AND METHODS

Experimental Design

The factors studied were the presence of cracks in enamel and enamel microabrasion. The response variable was the transenamel and transdentinal penetration of hydrogen peroxide, and the three basic principles of experimentation (repetition, randomization, and blocking) were observed. The sample had 72 teeth, 60 intended for the penetration study (n=20) and 12 for photomicrographs of the enamel surfaces (n=2).

Collection and Standardization of Specimens

Seventy-two experimental units were obtained from bovine incisors aged between 24 and 30 months. Those with stains on the enamel and/or excessive wear on the incisal were excluded.

The selected teeth were cleaned mechanically with periodontal curettes and received prophylaxis with pumice and water. Subsequently, they were fixed on a device attached to a drill platform bench (FGC-16 model, Ferrari, São Paulo, SP, Brazil). Cylinders were obtained from the middle third of the buccal surface (5.7 mm in diameter) with the aid of a diamond tip to cut glass (Diamond tip, 08 mm in diameter, Dinser Diamond Tools Ltda, Sacomã, SP, Brazil) and under constant irrigation.

The dentin surface was regularized through manual rotation movements using 600 grit aluminum oxide sandpaper (T469-SF-Noton, Saint-Gobam Abrasives Ltda, Jundiai, SP, Brazil)—until the specimens presented a thickness of 3.5 mm (approximately 1.3 mm and 2.2 mm of enamel and dentin, respectively), measured in a digital caliper (500 to 144 B, Mitutoyo South America Ltda, Suzano, SP, Brazil). The smear layer formed during grinding was removed by applying EDTA solution for one minute, and then the specimens were rinsed with deionized water.

Division of the Groups

The analysis of the enamel was done by stereomicroscopy (Stemi SV11, Carl Zeiss, Jena, Germany) at 45× to select sound and cracked enamel. After the selection and standardization of specimens, these

were divided into three groups (n=20). The 12 remaining specimens were used to analyze the enamel surface morphology using scanning electron microscopy (SEM).

Specimens were selected with no cracks for groups I and III. In group II, there were teeth with only cracks, with a length approximately that of the specimen diameter (between 4 and 5.7 mm).

The specimens of group I and II did not receive any treatment prior to bleaching. The specimens of group III received ten 15-second applications of the microabrasive Whiteness RM product (FGM Dental Products, Joinville, SC, Brazil). The product was applied with a rubber cup mounted in a 10:1 speed reduction contra angle. After the applications, the specimens were washed and dried, with each rubber cup used for only one specimen.

Preparation of Artificial Pulp Chamber

Each enamel/dentin disc was adapted individually to an artificial pulp chamber (APC), developed at the Laboratory of Experimental Pathology and Biomaterials of Araraquara School of Dentistry – UNESP.[20] Each APC was formed with two compartments: in the upper portion, an opening with a 8 mm diameter and, in the second compartment, an opening with a 6 mm diameter allowed for the appropriate positioning and lateral sealing of the specimen. The lower portion contained lateral perforations for circulation of the solution and was used to quantify the peroxide penetrating the specimen (Figure 1).

The specimens were positioned in the APCs between two silicone rings (5.60 mm inner diameter; 1.78 mm thick; Ref. OR 008 - Rodimar Rolamentos Ltda, Araraquara, SP, Brazil) and sealed with pink wax, restricting the lateral penetration of the bleaching agent (Figure 1).

Whitening Procedure

The specimens from all three groups were submitted to three in-office bleaching sessions using 0.04 mL of 35% hydrogen peroxide (Whiteness HP Maxx, FGM Dental Products). The product was deposited on the enamel and reapplied twice each session, for a total exposure time of 45 minutes per whitening session. All manipulation was performed in accordance with the manufacturer's instruction.

Quantification of H_2O_2 Penetration

For quantification of the H_2O_2 that penetrated the enamel/dentin discs, the artificial pulp chambers were individually placed in wells of acrylic cell culture plates. Each well was filled with 1 mL of acetate buffer solution and subsequently received APCs, already containing the dental fragments. Thus, the dentin surface remained in contact with the acetate solution during all bleaching procedures, and the diffused hydrogen peroxide became part of the same.

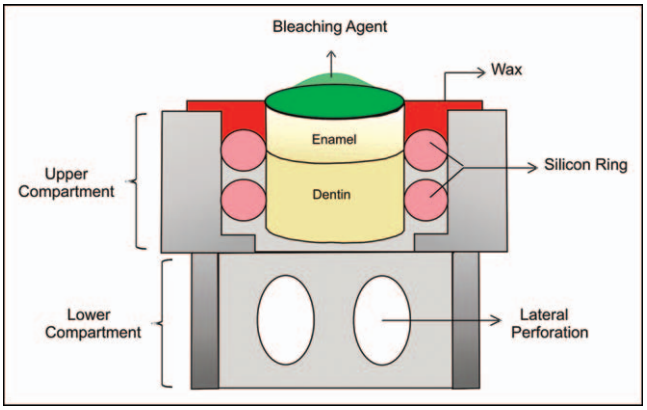

Figure 1. *Enamel/dentin disc positioned in the artificial pulp chamber.*

After the whitening procedure was done, 25 μL of acetate buffer solution was removed and mixed with 2.750 μL of distilled water, 100 mL of leucocrystal violet (0.5 mg/mL, Sigma Chemical Co, St Louis, MO, USA), and 50 μL of peroxidase (1 mg/mL, Sigma Chemical Co) and the solution was diluted to a final volume of 3 mL with distilled water.

This method is based on the reaction of hydrogen peroxide with leucocrystal violet, catalyzed by the peroxidase enzyme.[6,21-27] The coloring of the mixture varies in intensity according to the amount of peroxide. Thus, as the signal is proportional to the absorbance of the peroxide concentration, it is possible to assess indirectly the amount of peroxide that has penetrated the tooth surface and the solution contained in the wells.

Readings were done using ultraviolet visible reflectance spectrophotometer equipment (UV-2450, Shimadzu, Kyoto, Japan), 30 minutes after each bleaching session (Figure 2).

To obtain the calibration factor (CF) equivalent to the ratio of the concentration of the standard solution of hydrogen peroxide to its absorbance, the following equation was used:

$$CF = \frac{[Sample\ \ Solution]}{Absorbance}$$

The average calibration factor was used to calculate the hydrogen peroxide concentration contained in

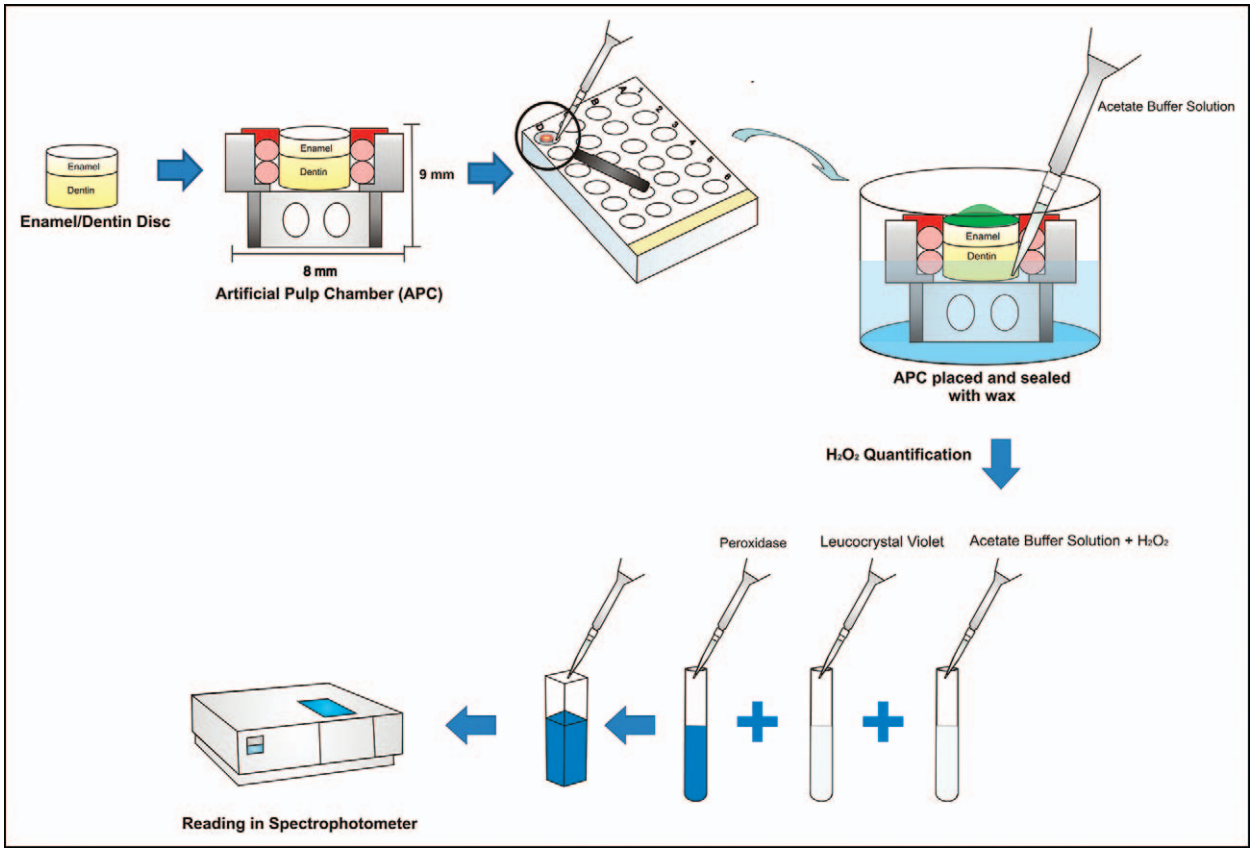

Figure 2. *Scheme of the sequence of the methodology employed in the quantification of hydrogen peroxide.*

each specimen. Data were tabulated and verified the normality and homogeneity of variance assumptions. Parametric tests were performed using analysis of variance (ANOVA) and Fisher's PLSD test, using the statistical program Stat View software, at a significance level of 0.05.

For illustrative purposes and to seek more plausible explanations for the results, the enamel surface morphologies of two specimens of each group were prepared for analysis using SEM (EVO HD LS-15, Carl Zeiss, Jena, Germany). The specimens were cleaned, dried, sputter coated, and photomicrographs were taken of the dental enamel under different conditions of study, at 3000× magnification.

RESULTS

The application of ANOVA showed significant differences in transenamel and transdentinal penetration of hydrogen peroxide at different substrate conditions ($p<0.0001$). The penetration was similar in the three bleaching sessions ($p=0.3624$), and there was no interaction between the substrate condition and the bleaching sessions ($p=0.5320$).

Table 1 shows that the quality of the substrate influenced the intensity of transenamel and transdentinal penetration of hydrogen peroxide, confirming higher hydrogen peroxide penetration in cracked teeth (GII). The microabraded enamel tooth (GIII) had intermediate values, and the control group (GI) always provided the greatest challenges for peroxide penetration.

DISCUSSION

Bovine teeth are convenient for laboratory study because they are easy to obtain in large quantities and have appropriate dimensions for artificial pulp chambers; bovine teeth also present uniform composition and have low variation in the experimental response.[23,28-31]

The methodology used in this study was proposed by Mottola and others[21] and is characterized by precision and selectivity of reagents prior to exposure to hydrogen peroxide. Thus, it is important to note that the values obtained show only the concentration of hydrogen peroxide in the solution

Table 1: *H_2O_2 Average in μg (Standard Deviation) Accompanied by the Statistical Groups, Verified by Applying the Fisher's PLSD Test**

	Sound	Cracked	Microabrasioned
1[a] Session	6.01 (0.93) Ac	9.44 (1.71) Aa	7.81 (1.78) Ab
2[a] Session	6.12 (1.26) Ac	9.81 (1.81) Aa	8.69 (1.47) Ab
3[a] Session	6.65 (0.72) Ac	9.28 (1.68) Aa	8.06 (1.12) Ab

** Statistical groups are denoted by letters: capital letters in the columns and lowercase letters in the rows ($p<0.05$). The amount of H_2O_2 is contained in 3 mL of solution.*

and not the other substances produced by the decomposition of the bleaching agents.

Even though vital tooth whitening is considered a conservative treatment,[1,2] there are concerns regarding the use of highly concentrated products because human teeth treated with these substances exhibit high levels of sensitivity[8,9] and demonstrate irreversible pulp damage.[20,32-35]

Even when considering enamel to be semipermeable, some clinical conditions can lead to compromised biologic safety of the bleaching procedure. The amount of peroxide that reaches the pulp-dentin complex can be altered to interfere with pulpal health.[8,20,34]

Results show that the presence of cracks and the enamel microabrasion procedure allowed greater penetration of hydrogen peroxide when compared to sound teeth.

In this context, the aim of the enamel microabrasion and the removal or reduction of chromatic changes or irregularities of the enamel surface may represent a health concern for the pulp-dentin complex. Once the microabrasive action changes the enamel histomorphology, the tooth becomes more permeable.[15,17,18,19] In these cases, there is a need for complementing the esthetic treatment with bleaching because yellowing of the dentin tissue becomes more apparent.[19] In most cases, the combination of these techniques is a treatment plan of relatively low cost and provides a great possibility to obtain optimum esthetic results. However, until now, there were no obvious possible biologic implications that bleaching can cause after microabrasion.

It is believed that rubbing the abrasive on the destructured enamel, caused by the acid pH of the product, alters its surface layer and perhaps the enamel permeability, making it more susceptible to the bleaching agent's penetration. Additionally, this acid-abrasive process reduces the enamel thickness,[15,17,18,19] and the obstacles to peroxide penetration.

In the same way, the enamel often presents cracks which, depending on length, may be continuous with the pulp-dentin complex.[36-38] This fact justifies precautions when dental products could inadvertently be applied to the adamantine substrate.

A crack is a rupture of the tissue into two or more parts and may involve different dental tissues or be restricted to a small portion of the enamel surface.[37] In the present study, we selected samples showing visible cracks with an extension varying between 4 and 5.7 mm. As during the sample preparation, there was no enamel/dentin separation; the cracks represented the presence of fissures only in the enamel structure, not across the dentin-enamel junction. But, even when taking the necessary precautions in the standardized sample, the high penetration rates observed in cracked specimens show that these pathways greatly facilitate the passage of bleaching agents that quickly reach the dentinal tubules. Perhaps this occurrence explains the frequent reports and complaints about the increase of tooth sensitivity during bleaching in patients with this clinical condition.[10,13,39,40]

Because they are difficult to diagnose, cracks can be neglected or overlooked by some professionals, who are often unaware of the consequences of the free passage of dental products for the pulp-dentin complex.

These results underscore the importance of maintaining intact enamel before the start of treatment. In cases using both microabrasion and whitening, tooth whitening should occur first because enamel changes that indicate microabrasion may be imperceptible after bleaching treatments. Furthermore, and most important, the presence of the sound enamel will be more effective in modulating the levels of peroxide penetration into the pulp tissue than microabraded enamel. On the other hand, careful clinical examination of cracks is important; cracks can be identified and confirmed by the naked eye or simply via macrography, microscopy, or transillumination.[13,41] These structural defects should be obliterated before

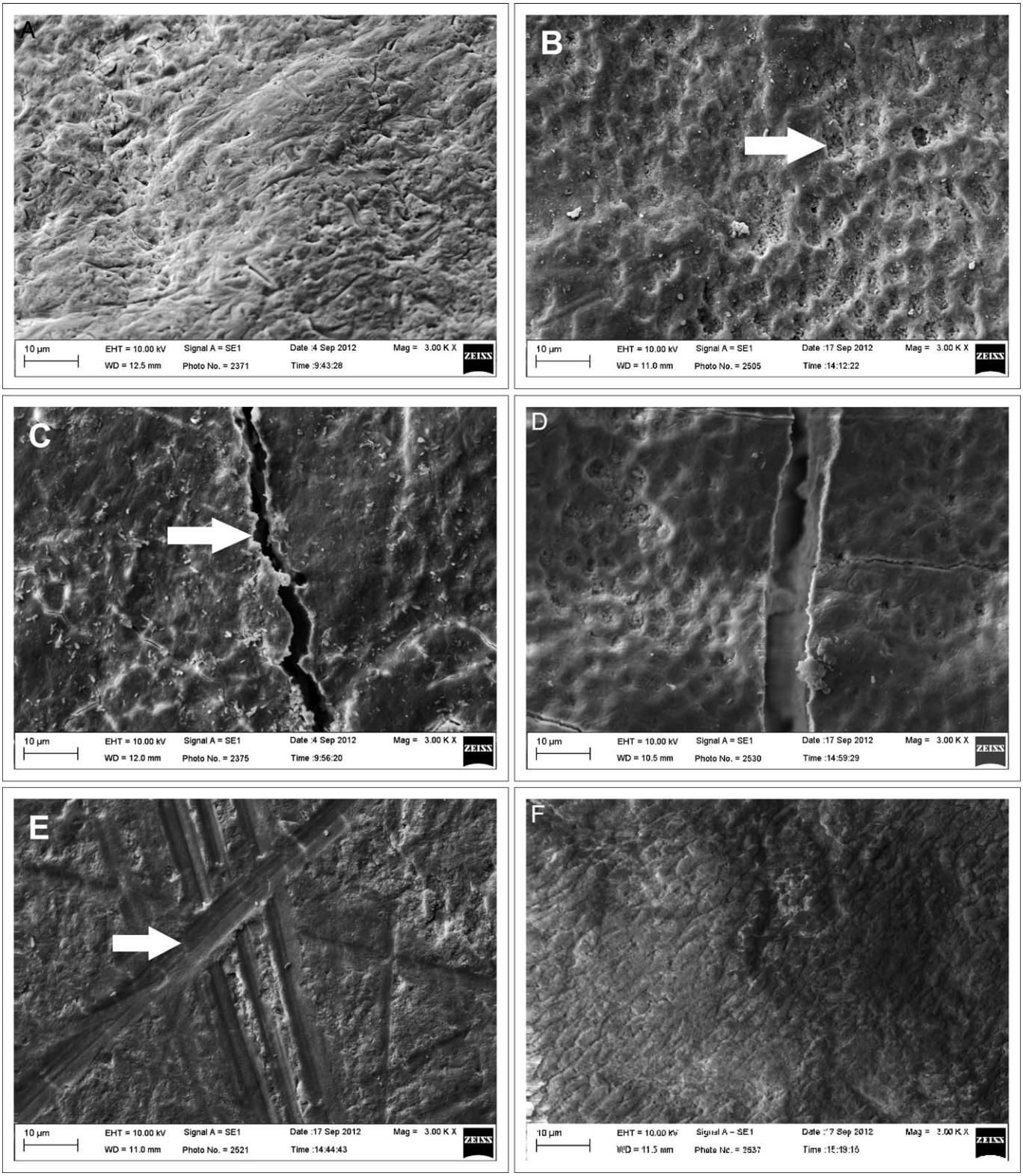

Figure 3. *(A): Specimen from the control group showing the characteristic diffusion enamel channels (3000×). (B): After the bleaching treatment, note the removal of part of the aprismatic layer and partial dissolution of the prisms (arrow) (3000×). (C): Specimen showing cracks in the enamel (arrow). Compared to the remaining structure, this structural defect is important in the formation of a continuous solution with the dentin pulp complex (3000×). (D): After the bleaching procedure, besides the crack, there is a partial dissolution of the aprismatic layer and increased porosity, forming new diffusion enamel channels for peroxide (3000×). (E): Microabrasioned enamel tooth, with grooves in the surface (arrow) (3000×). (F): Enamel surface previously microabrasioned exposed to 35% HP. Note the removal of the grooves and the view of the enamel prisms openings (3000×).*

starting the bleaching treatment. For this purpose, there are clinical reports of utilizing total-etch adhesive systems, self-etch adhesives, and pit and fissure sealants.[42,43]

The results of *in vitro* studies cannot be directly extrapolated to clinical situations. The penetration of hydrogen peroxide in the bovine teeth was less when compared with that in human teeth. The thickness of the aprismatic enamel layer in bovine teeth and reduction in diameter of the tubules as they approach the pulp chamber are different from what occurs in human dentin.[22] Moreover, the presence of intrapulpal pressure, cytoplasmic processes of odontoblasts, the constant demineralization/remineralization cycles, and the presence of saliva may decrease peroxide diffusion.[34,44-47] Thus, more studies evaluating these different conditions are needed to establish a secure protocol.

CONCLUSIONS

Considering these results, enamel microabrasion makes this substrate more susceptible to hydrogen peroxide penetration. In addition, teeth presenting cracks in the enamel allow greater hydrogen peroxide penetration during in-office whitening.

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Conflict of Interest

The authors 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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Detection of Marginal Leakage of Class V Restorations *In Vitro* by Micro–Computed Tomography

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Y Li

Clinical Relevance

Micro–computed tomography can provide *in vitro* nondestructive detection of leakage around a composite restoration to display entire three dimensional dentin leakage patterns with accuracy comparable to that of the conventional dye-penetration method.

SUMMARY

This *in vitro* study evaluated the efficacy of micro–computed tomography (CT) in marginal leakage detection of Class V restorations. Standardized Class V preparations with cervical margins in dentin and occlusal margins in enamel were made in 20 extracted human molars and restored with dental bonding agents and resin composite. All teeth were then immersed in 50% ammoniacal silver nitrate solution for 12 hours, followed by a developing solution for eight hours. Each restoration was scanned by micro-CT, the depth of marginal silver leakage in the central scanning section was measured, and the three-dimensional images of the silver leakage around each restoration were reconstructed. Afterward, all restorations were cut through the center and examined for leakage depth using a microscope. The silver leakage depth of each restoration obtained by the micro-CT and the microscope were compared for equivalency. The silver leakage depth in cervical walls observed by micro-CT and microscope showed no significant difference; however, in certain cases the judgment of leakage depth in the occlusal wall in micro-CT image was affected by adjacent enamel structure, providing less leakage depth than was observed with the microscope ($p<0.01$). Micro-CT displayed the three-dimensional image of the leakage around the Class V restorations with clear borders only in the dentin region. It can be concluded that micro-CT can detect nondestructively the leakage around a resin composite restoration in two and three dimensions, with accuracy comparable to that of the conventional microscope method in the dentin region but with inferior accuracy in the enamel region.

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INTRODUCTION

Marginal adaptation of dental restorations is an important factor that influences the longevity of

dental restorations. Poor marginal adaptation will result in microleakage along the tooth-restoration interface, leading to marginal staining and increasing the risk of postoperative sensitivity and secondary caries.[1] Microleakage has been defined as passage of substances, such as saliva, ions, compounds, or bacterial by-products, between a cavity wall and the restorative material.[2] The investigation of microleakage is, therefore, important in the assessment of restorative materials. A variety of *in vitro* methods have been introduced into the investigation of microleakage, including compressed air, electrochemical approaches, bacteria infiltration, scanning electron microscopy (SEM), and perhaps most common of all, the dye-penetration method.[2,3] The conventional dye-penetration method, also known as the microscope method, typically involves soaking a restored tooth in a dye solution, cutting through the center of the restoration, and assessing the leakage visually detectable on the section under a microscope. The extent of leakage may be quantified using a length scale, but it is often more subjectively evaluated using a system whereby the degree of leakage is graded by operators using a predetermined range of values.[4] However, this methodology has been known to have a number of shortcomings, among which the major issue is that the results obtained are two-dimensional (2D) in nature and do not take the whole tooth-restoration interface into account, as only a few sectioned planes are measured for leakage. Furthermore, some studies have shown that the dye leakage in different sections taken from different places on the restoration varied significantly.[5]

Although some other methods, such as the compressed air method,[6] are capable of producing results revealing the whole tooth-restoration interface, the path and location of leakage cannot be demonstrated, which is problematic because these are helpful for understanding the mechanism and etiology of microleakage and its predilection site.

To overcome this drawback, Gale and others,[7] Youngsen,[8] and Iwami and others[9] reported a three-dimensional (3D) method that revealed the dye penetration at the adhesive interface of resin restorations. With their method, images of the sectional surface of the resin restorations are taken with continuous reductions, and 3D images are finally created from the obtained images using computer software. However, this method is destructive and time consuming, and the 3D images obtained are rough because of the low spatial resolution (more than 100~300 μm between two sections).

Micro-CT uses X-rays to create cross sections of a 3D object that can be used to recreate a virtual model with a spatial resolution at a micron level without destroying the original specimen. Micro-CT has been used widely in material research to detect the inner structure of materials.

Kakaboura and others[10] investigated the gap along the interface between resin composite restorations and tooth structure using micro-CT. To display the effects of polymerization contraction of resin composite on the gap, they did not apply any conditioner and bonding agents prior to filling the cavity with the composite (which was not in agreement with clinical protocol). Consequently, the gap imaged by the micro-CT would be different from the true situation. Sun and others[11] investigated the volume contraction of cylindrical fillings of resin composite in a model cavity prepared in a PMMA block using the micro-CT. They used rhodamine B dye solution as a tracer to penetrate the gap between the resin composite restoration and the cavity wall and referred to the stained area as microleakage. Both of these two studies demonstrated the gap leakage created by resin polymerization contraction but did not take adhesion into account. In fact, according to the definition of microleakage,[3] it is caused not only by gaps resulting from contraction but also by defects located in the bonding interface, especially in the hybrid layer—the concept of nanoleakage introduced by Sano and others,[12] which even shows gap-free margins around restorations.

It is possible to use micro-CT to detect the leakage around a bonded restoration. However, it is unclear whether the micro-CT is comparable to the conventional dye-penetration method in displaying the leakage. Therefore, in this *in vitro* study, we used the micro-CT and dye-penetration method to examine the leakage around the same Class V resin composite restorations and compared the difference in the results in leakage depths obtained by the two methods in order to evaluate their equivalency in measuring marginal leakage. In addition, the efficacy of micro-CT in demonstrating the 3D shape of the leakage was evaluated.

MATERIALS AND METHODS

Twenty caries-free third molars were selected within six months of extraction. After surface debridement with a hand scaling instrument and cleaning with a

rubber cup and pumice, a standardized Class V cavity was prepared on the buccal or lingual surface of each tooth (3 mm in diameter and 1.5 mm in depth), with the cervical margin located in the dentin and the coronal margin in the enamel. The cavities were prepared first using a cylindrical diamond bur with a high-speed handpiece and air-water coolant spray and were then finished using a carbide bur with a straight flat fissure head without cross-cuts.

The cavities were then checked for cracks at the margins using a stereomicroscope. This step is necessary to eliminate those cavities with defects that may allow tracer solution to ingress through microscopic spaces of the tooth/restoration interface, thereby giving false-positive results. The teeth were randomly assigned to four groups (n=5) for four adhesive systems, as follows: 1) Prime & Bond NT (Lot No. 506001515; Dentsply, Kanstanz, Germany), 2) Clearfil SE Bond (Lot No. 71167; Kuraray, Okyama, Japan), 3) Adper Prompt (Lot No. 255379; 3M ESPE, St Paul, MN, USA), and 4) iBond (Lot No. 10069; Hereaus, Hanau, Germany). The adhesive systems were applied according to the manufacturers' instructions, and the cavities were then restored with a resin composite (Clearfil AP-X, Lot No. 1089AA, Kuraray) and cured for 40 seconds with a light at 800 mW (LED D, Woodpecker Co, Guilin, China). After the teeth were stored in distilled water for 24 hours at 37°C, the surface of each restoration was finished with a diamond-coated abrasive bur to remove possible resin flash overlaying the enamel so that a butt joint was formed; this was followed by finishing with a rubber cup with abrasive paste and then polishing with polishing disks (Soflex, 3M ESPE).

To prevent silver penetration in areas other than the exposed margins, the root apices were etched and sealed with a light-curing fissure sealant (Clinpro, 3M ESPE). The tooth surfaces were then sealed with two layers of nail varnish to be within 1 mm of the restoration margins.

All of the teeth were immersed in 50% ammoniacal silver nitrate solution in the dark for 12 hours, rinsed with running water for two minutes, immersed in photo-developing solution, and exposed to light for eight hours. Then, the teeth were cleaned with a toothbrush and polished slightly with Soflex disks to remove silver depositions on the surface.

After being placed and fixed into the specimen holder, each tooth was scanned individually using a micro-CT (Inveon, Siemens, Germany) at a resolution of 20 μm with an integration time of 2500 ms. The micro-focus X-ray source was set at 80 kV and 500 μA. After completion of the *x–y* scans, the 2D and 3D images of each restoration were reconstructed using image analysis software of the instrument—Inveon CT Research Workplace. The leakage depths along the cervical wall and coronal wall of each restoration, including their respective extensions at the axial wall, were measured on the section image scanned longitudinally through the center of the restoration. In addition, the 3D image of each restoration and the leakage around the restoration were reconstructed with the software. Furthermore, to display the leakage more clearly in 3D, the images of silver leakage were picked up digitally according to a radiopaque value that was just higher than the value of enamel using the software.

All teeth were then sectioned longitudinally through the center of the restoration in a buccolingual direction with a slow-speed diamond saw (SYJ-150, MTI Corporation, China) under water-cooling. The thickness of the cutting blade was 0.2 mm. The black leakages along the cervical and coronal walls, as well as their extensions in the axial wall of each restoration on the section, were photographed and measured for the leakage depth with a measuring microscope at 10× magnification.

Statistical Analysis

Statistical comparisons were made between the results of the micro-CT and microscope methods for equality in measuring the leakage depths. The data for leakage depths at the coronal and cervical walls of each restoration, measured by the micro-CT and microscope, were compared with the Wilcoxon paired-rank test. All data were submitted for statistical analysis at the $p < 0.05$ level of significance.

RESULTS

Table 1 lists the leakage depths along the coronal and cervical walls of each restoration measured with the micro-CT and microscope. The Wilcoxon paired-rank test revealed no significant difference between the two methods in measuring the depth of leakage at the cervical margin, but there was a significant difference ($p<0.01$) at the coronal margin, with the depths from micro-CT being lower than those from the microscope.

Figure 1 shows images of the leakage at the mid-longitudinal sections of four typical restorations demonstrated with the micro-CT and microscope. It

Table 1: *Leakage Depths in Cervical and Coronal Margins Measured With Micro–Computed Tomography (micro-CT) and Microscopy*

Bonding Agents	Restoration	Leakage Depth, mm			
		Cervical[a]		Coronal	
		Micro-CT	Microscope	Micro-CT	Microscope
Prime & Bond NT	1	3.22	3.14	0	0.06
	2	0.66	0.69	1.32	1.22
	3	1.19	1.11	0	0
	4	0.09	0.14	1.01	1.22
	5	1.43	1.44	0	0.30
Clearfil SE Bond	1	0	0	0.24	0.33
	2	0	0	0.74	0.99
	3	0.14	0.13	0.23	0.26
	4	0.45	0.42	0.2	0.26
	5	0.18	0.17	0.36	0.45
Adper Prompt	1	1.11	1.06	0.47	0.52
	2	1.51	1.51	0.52	0.66
	3	1.23	1.23	0.86	1.51
	4	1.62	1.66	0.16	0.18
	5	1.48	1.49	0.7	0.91
iBond	1	0.22	0.19	0.33	0.9
	2	1.03	1.03	0	0.98
	3	0.12	0.15	0	0.26
	4	1.47	1.33	0.82	0.86
	5	0.23	0.24	0	0

[a] *Including the extension of the leakage in the axial wall.*

is obvious that silver leakage depths in the cervical margin of the same restoration presented in the micro-CT image and microscope image are comparable, whereas the leakage depths in the coronal margin of the same restoration are different in some restorations, with deeper leakage visible in the microscope images compared with those in the micro-CT image, such as those illustrated in Figures 1e and f.

Figure 2 shows 3D images of the leakage around four restorations. Obviously, most restorations presented more leakage in the cervical region than in the coronal region in terms of both depth and area (Figure 2a). However, the leakage did not always occur in the cervical region or in the coronal region. In contrast, some leakage occurred primarily in the mesial or distal wall of the cavity (Figure 2e,g), and some even presented no leakage in the center of the cervical region (Figure 2e). In addition, some leakage extended to the axial wall from the mesial or distal wall of the cavity (Figure 2c). The leakage expanded in different shapes, with some leakage extended from the external surface into the cavity through certain passages, as opposed to enlargement of leakage area by continuous degrees from the outside into the inside, such as the leakage marked with an arrow in Figure 2, in which the leakage obviously extends from two sides.

DISCUSSION

The conventional dye-penetration method has been used widely to determine marginal adaptation and is listed as a standard method by the International Organization for Standardization.[13] To evaluate the efficacy of micro-CT in the determination of leakage, the present study compared the leakage depth along the cervical and coronal walls in the mid-longitudinal sections of each restoration obtained with a micro-CT with those measured using the conventional dye-penetration method. The results indicate that leakage depths measured with the micro-CT and microscope at cervical walls of each restoration were comparable, which indicates that micro-CT is reliable in detecting marginal leakage located in dentin when using silver as a radiographic contrast agent.

It is important for the methodology of micro-CT that the radiographic contrast agents used to display

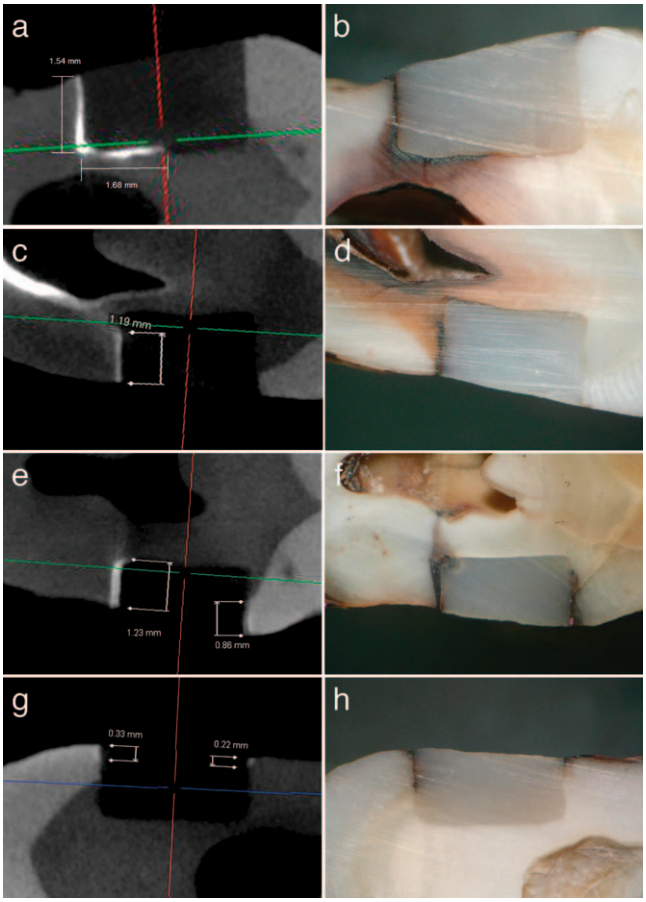

Figure 1. *Marginal leakage on the middle longitudinal sections of four restorations. (a, c, e, and g) Images of the micro-CT; (b, d, f, and h) corresponding images from the microscope after cutting.*

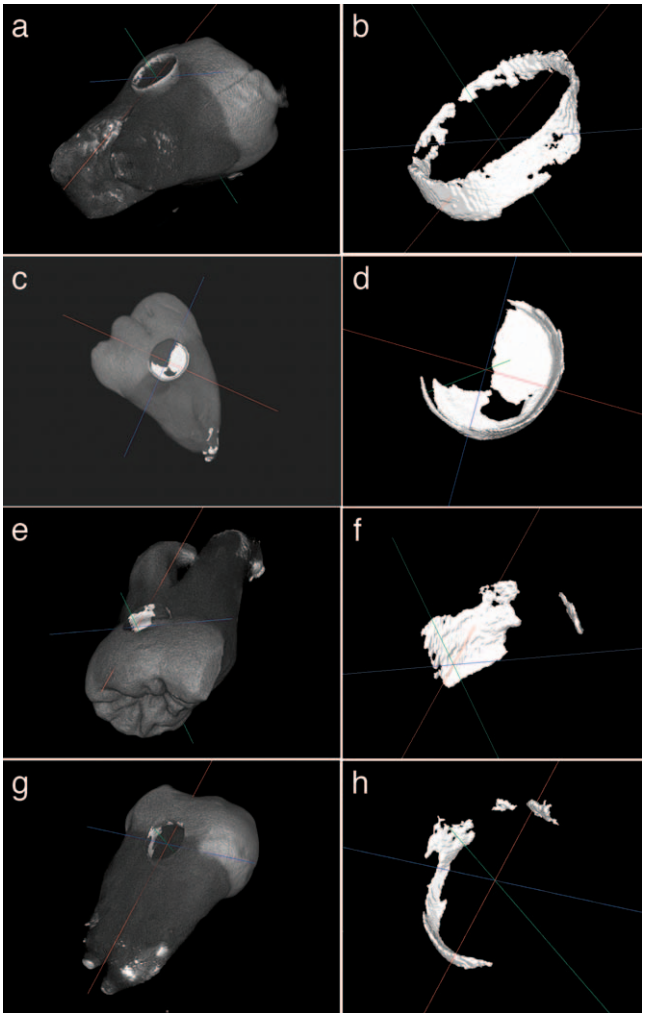

Figure 2. *3D images of silver leakages around four Class V restorations. (a, c, e, and g) Leakage in the cavity after removal of restorations digitally; (b, d, f, and h) corresponding images of silver leakage after removal of both restorations and tooth structures digitally.*

leakage should be highly radiopaque in relation to the restorative material and tooth structure so that even a small amount of leakage can be differentiated. The two main factors contributing to a material's radiopacity are atomic number and density.[14] Silver has a much higher atomic number than do the elements that exist in tooth hard tissues and composite resin fillings; thus, silver is capable of presenting a good radiopaque contrast when it is dense. Dentin contains less hydroxyapatite compared to enamel, which makes it less radiopaque than enamel and much less radiopaque than silver and thus provides a good contrast to silver leakage. Accordingly, silver solution, such as 50% ammoniacal silver nitrate, has broadly been used as a tracer in nanoleakage studies on dentin bonding interface under transmission electron microscopy or SEM.[12,15] Therefore, it was easy to determine the border of silver leakage in the dentin region in the present study.

As mentioned above, the density of silver leakage affects its radiopacity. In the present study, heavy silver leakage presented a good radiopaque contrast to enamel structure and demonstrated a clear border in the enamel region. However, slight silver leakage presented a decreased radiopacity and thus a poor radiopaque contrast to enamel structure, leading to difficulty in identification of the border of silver leakage on the CT image. Even a small portion of some slight leakage presented a similar radiopacity to the adjacent enamel structure and therefore cannot be identified, which resulted in a measured depth of silver leakage that was less than its true depth. To reconstruct the 3D image of silver leakage, the software picked up the silver leakage according

to a radiopaque value that was just higher than the value of enamel. Thus, some peripheral parts of the silver leakage next to the enamel structure cannot be picked up as a result of their lower radiopaque value, which resulted in a 3D image of the leakage that was smaller than it should have been.

To improve the differentiability of leakage in the CT method, there is a need for a better leakage tracer that presents a preferable radiopaque contrast to enamel structure. In addition, micro-CT with a higher resolution can present a clearer margin of leakage, which is conducive to the identification of silver leakage.

To present accurately the leakage around a restoration, the leakage tracer should be nondestructive of tooth tissue. Previous studies[16] demonstrated that the nonbuffered silver nitrate solution at pH 3.5 is highly acidic and thus erosive to tooth structures, producing its own path into the tooth/restoration interface. However, the pH of silver nitrate solution can be buffered with ammonia to a pH value of about 7; therefore, a buffered silver nitrate solution is suitable to trace the leakage around a restoration.

One of the great advantages of micro-CT is that it is nondestructive to the specimens tested because there is no need for cutting, which allows the specimens to maintain their original state after measurement and to be further used in other measurements or studies (eg, for observation under a microscope or a SEM). Another advantage of micro-CT is its ability to display the leakage at any section and to reconstruct a 3D image of the leakage around a restoration. Furthermore, the leakage can be measured quantitatively in length, area, and volume using analysis software. The 3D image of any structure in the restored tooth, such as the leakage or the resin composite restoration, can be picked up digitally to display individually, giving a realistic view.

The 3D mapping of the leakage around Class V restorations in this study reveals that the leakage occurs more often and is more severe at the cervical wall than at the coronal wall, which is in agreement with what was previously reported by Pickard and Gaynford[6] and Gale and others[7] using the conventional dye-penetration method. However, the leakage observed on the mid-longitudinal sections is not inadequate to characterize the entire leakage around the restoration, because the leakage around a restoration does not always develop evenly around the cavity wall (eg, the leakage shown in Figure 2a-d). Some leakage even occurs locally in the mesial or distal wall of the cavity, such as the leakage shown in Figure 2e, in which no leakage is detectable at the cervical wall on the mid-longitudinal section. These findings are in agreement with those of Raskin and others,[17] who tested how the number of sections affected the maximum depth of tracer penetration and revealed that microleakage was seldom uniformly distributed. To characterize the degree of the leakage around a restoration, the percentage of the leakage area to the whole area of the cavity wall will be a more meaningful, quantitative index because it takes the whole tooth-restoration interface and the volume of a restoration into account.

It is unclear how the leakage develops and penetrates along the interface, and, thus, a 3D image of the leakage would be helpful to find the answer. As mentioned above, microleakage is the passage of substances such as saliva, ions, compounds, or bacterial by-products, which can be created not only by the contraction gaps of restorative materials but also by the structure defects located within the joint interface between the restoration and the dental structure.[12] The study by Pushpa and Suresh[18] confirmed that the marginal microleakage of composite restorations bonded with one-step self-etch adhesives is closely related to the hydrophilicity of adhesive layer, which is able to generate structure defects in the bonding interface.[19] In this case, the leakage observed may be created mainly by the structure defects within the bonding interface and therefore shows no correlation to the polymerization contraction of the restorations.[20] Accordingly, it is likely that the leakage in the present study demonstrates more action of the structure defects and less action of polymerization contraction of the restoration. Regardless, all of the leakage can be marked by a tracer such as silver solution.

The leakage depth observed varied considerably within some groups; a possible reason for this is the different storage period of the teeth before testing, which varied from three days to six months. There is increasing evidence that storage condition and storage time of the dentin after extraction may influence dentin permeability, adhesion to dentin, and, consequently, marginal leakage.[21-23] On the other hand, the variation in leakage depth provides an additional opportunity to compare the conformity of the results of micro-CT to those of the conventional dye-penetration method in samples with different degrees of leakage.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following are concluded:

- Micro-CT can be used to detect nondestructively the leakage around a composite restoration in 2D and 3D when using a solution of ammoniacal silver nitrate as the tracer.
- Micro-CT has accuracy that is comparable to that of the conventional dye-penetration method in terms of measuring the leakage in the dentin region, but it offers inferior accuracy in the enamel region as a result of the lower radiopacity contrast of silver tracer to enamel.
- The conventional dye-penetration method has the disadvantage of being unable to display the entire leakage around a restoration, and its results are inadequate in terms of characterizing the leakage around a restoration.
- The leakage around a Class V composite restoration is diverse in shape, location, and distribution (rather than always distributing evenly around the cavity wall).

Conflict of Interest

The authors 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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Fracture Strength and Fracture Patterns of Root-filled Teeth Restored With Direct Resin Composite Restorations Under Static and Fatigue Loading

NA Taha • JE Palamara • HH Messer

Clinical Relevance

Root-filled teeth suffer substantial loss of tooth structure from restorative and endodontic procedures. A direct adhesive restoration will help preserve the remaining tooth structure as long as it provides enough strength and marginal integrity.

SUMMARY

Aim: To assess fracture strength and fracture patterns of root-filled teeth with direct resin composite restorations under static and fatigue loading.

Methodology: MOD cavities plus endodontic access were prepared in 48 premolars. Teeth were root filled and divided into three restorative groups, as follows 1) resin composite; 2) glass ionomer cement (GIC) core and resin composite; and 3) open laminate technique with GIC and resin composite. Teeth were loaded in a servohydraulic material test system. Eight samples in each group were subjected to stepped fatigue loading: a preconditioning load of 100 N (5000 cycles) followed by 30,000 cycles each at 200 N and higher loads in 50-N increments until fracture. Noncycled teeth were subjected to a ramped load. Fracture load, number of cycles, and fracture patterns were recorded. Data were analyzed using two-way analysis of variance and Bonferroni tests.

Results: Fatigue cycling reduced fracture strength significantly ($p<0.001$). Teeth restored with a GIC core and a laminate technique were significantly weaker than the composite group (379±56 N, 352±67 N vs 490±78 N, $p=0.001$). Initial debonding occurred before the tooth underwent fracture. All failures were predominantly adhesive, with subcrestal fracture of the buccal cusp.

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Conclusions: Resin composite restorations had significantly higher fracture strength than did other restorations. Fatigue cycled teeth failed at lower load than did noncycled teeth.

INTRODUCTION

Root-filled teeth suffer substantial loss of tooth structure as a result of caries and subsequent restorative procedures, making them vulnerable to fracture if they are not adequately restored.[1] Given their low cost, resin composites are becoming increasingly common in the restoration of root-filled teeth, to an extent replacing coronal coverage restorations. Concerns thus arise with regard to the strength of such restorations, and the problem of marginal leakage and its sequelae must also be considered. The use of low shrinkage composites and new generations of dentin bonding agents has been investigated,[2-4] and in terms of fracture resistance, favorable results have been reported in both experimental and clinical studies.[5-7] However, the problem of marginal leakage continues to be a concern. Hence, different liners, including flowable composites, resin-modified, and conventional glass ionomer cements (GIC), have been investigated both as a core and in a laminate ("sandwich") technique and have been found to be beneficial in reducing marginal leakage.[3,8-11]

Cusp fracture of restored teeth with or without root canal therapy is a common finding clinically. The most common cause has been reported to be a high impact force as a result of biting on a hard object, with tooth anatomy and type and size of restoration being contributing factors.[12] Fatigue-related failure is also a major concern,[13] with cuspal fracture occurring after prolonged function. Experimentally, cyclic fatigue might have more drastic effects on fracture strength than a static load as a result of the initiation and propagation of cracks within tooth structure and restorations.[14,15] Fatigue fracture can occur at the point at which maximum stress occurs, which in the case of adhesive restorations is the tooth-restoration interface.[16]

Fracture resistance of teeth has been used as a measure of the effect of cavity preparation and restoration on tooth strength. Both destructive and nondestructive techniques and the creation of mathematical models for analyzing stress distributions have been used.[17-19] The clinical relevance of the static load to failure approach is questionable, as the load usually applied in these studies is well above the natural biting force. Variable numbers of load cycles at physiological loads preceding ramped load to fracture are commonly included to simulate normal oral function. Teeth are then subjected to static loading to fracture. Typically, however, the number of cycles represents only weeks or months of normal chewing cycles. Nevertheless, for comparison between restorative techniques and materials, static loading with or without cyclic loading can still be considered a valid approach.

The alternative to static load is fatigue testing, in an attempt to represent physiological mastication and fatigue failure of teeth or restorations. The number of cycles to failure at physiological loads (up to 100 N) is extremely high and makes fatigue testing of restored teeth impractical in many instances.[20] As an alternative, stepped fatigue loading, with a progressively increasing load for a specified number of cycles, is being used for fatigue testing of teeth.[21,22] A stepped load protocol was applied in this study; this protocol could be considered as an adequate compromise between the classic ramped load and the time-consuming conventional fatigue test (high number of cycles at low load).

The aim of the study was to assess the fracture resistance and fracture patterns of root-filled maxillary premolars with similar cavity design and three different direct restoration techniques using resin composite under static and fatigue loading.

MATERIALS AND METHODS

Tooth Selection and Mounting

Forty-eight extracted intact noncarious maxillary premolars of similar size (as measured from both bucco-lingual and mesio-distal directions of the occlusal surface of the crown using a digital caliper) were used. The selected teeth were mounted vertically in epoxy resin in polyvinyl chloride plastic rings without a simulated periodontal ligament, since in a previous study,[23] a simulated periodontal ligament (PDL) did not influence fracture strength under these conditions of testing. The epoxy resin mounting extended to 2 mm below the cemento-enamel junction (CEJ) to simulate the alveolar bone level. The project was approved by the Ethics in Human Research Committee of the University of Melbourne. Teeth were stored in 1% chloramine T solution in distilled water (pH=7.8) (Sigma-Aldrich Co, St Louis, MO, USA) for two weeks.

Cavity Preparations

Extensive MOD cavities, plus endodontic access with the proximal axial walls removed, were prepared as previously described,[23] using a tungsten carbide

round-ended fissure bur (Komet H21R, Brasseler, Lemgo, Germany) in a high-speed handpiece with water coolant. The bucco-lingual width of the occlusal isthmus was one-third of the width between buccal and lingual cusp tips, and the bucco-lingual width of the proximal box was one-third of the bucco-lingual width of the crown. The gingival floor of the box was 1 mm coronal to the CEJ; total depth of the proximal box occluso-gingivally was 6 mm. The cavosurface margins were prepared at 90°, and all internal angles were rounded. Endodontic access included the removal of all dentin between the proximal box and the pulp chamber.

Root canals were prepared using the ProTaper rotary nickel-titanium system (Dentsply, Maillefer, Ballaigues, Switzerland) with 1% NaOCl irrigation between instruments and a final flush with 17% ethylene diamine tetraacetic acid solution and root filled using gutta percha and AH Plus root canal sealer (Dentsply, Maillefer Detrey, Konstanz, Germany). Gutta percha was removed to 2 mm below the CEJ. Excess sealer was removed with a cotton pellet moistened with alcohol.

Restoration

The teeth were divided randomly into three groups of 16 teeth each using a random numbers table and were restored as follows (Figure 1).

Group 1 (Resin Composite)—The entire cavity preparation was etched with 37% phosphoric acid (Super Etch, SDI Limited, Bayswater, Australia; batch No. 030648) for 20 seconds, rinsed with air-water spray for 10 seconds, and blot dried (wet bonding in accordance with the manufacturer's instructions). A bonding agent (Adper™ Single Bond, 3M ESPE, St Paul, MN, USA; lot No. 184141) was applied and light-cured for 20 seconds, and the cavity was incrementally restored with OD3 shade resin composite with a matrix band in place (Glacier, SDI Limited;batch No. 071089). Three increments were placed and cured using a LED light-curing source (Bluephase C8, CE Ivoclar, Vivadent AG, F1-9494 Schaan, Liechtenstein) at an intensity of 800 mW/cm^2 for 40 seconds. The first increment was packed into canal orifices and both proximal boxes to a depth of approximately 1 mm. The last two increments covered the entire mesio-distal and bucco-lingual width of the cavity. Restorations were finished and polished using a flame-shaped diamond bur (Komet Dental, Brassler, Germany) and Sof-Lex finishing discs (3M ESPE).

Group 2 (GIC Core and Resin Composite Restoration)—Prior to restoration with composite, a 10% polyacrylic acid dentin conditioner was applied for 10 seconds and rinsed for five seconds, and then a conventional GIC base (Fuji VII, lot No. 0811111; GC Corporation, Tokyo, Japan) was placed above the gutta percha to reproduce the floor of a MOD cavity. Acid etching and the bonding agent were then applied to the cavity wall and over the GIC, and the teeth were then restored with resin composite, as in Group 1.

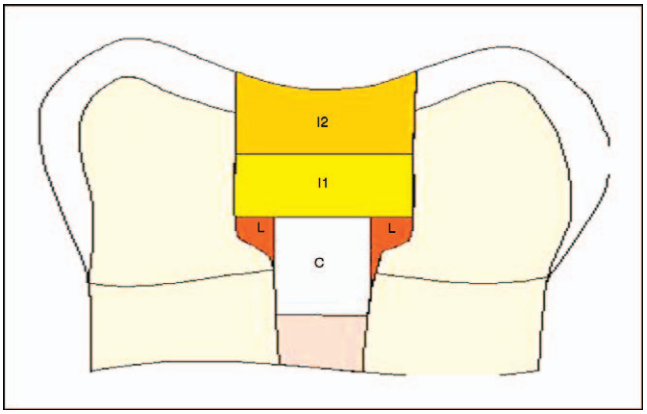

Figure 1. *A diagram representing the three restoration techniques. C (shown in gray) represents the GIC core, inserted into the coronal part of the canal space. The restoration shown in the orange area (L) plus the gray area shows the area filled when a laminate restoration was used, while the coronal resin composite placed in two increments (I1 and I2) is shown by the coronal part of the cavity.*

Group 3 (Open Laminate Technique and Resin Composite Restoration)—Prior to restoration, a 10% polyacrylic acid dentin conditioner was applied for 10 seconds and rinsed for five seconds and then a 1-2 mm glass ionomer base (Fuji IX, lot No. 0902245; GC Corporation) was placed above the gutta percha and into the proximal boxes to a thickness of 1.5-2 mm at the proximal surfaces. The teeth were then restored with two increments of resin composite, as described above.

After restoration, teeth were stored in an incubator at 37°C in 100% humidity for 24 hours before testing. Teeth in each restorative group were then randomly allocated to either static or fatigue loading subgroups (eight teeth each).

Static and Fatigue Loading

All teeth were subjected to fracture testing using either a static load or a stepped cyclic loading sequence.[22] Loading was at 45° to the long axis of the tooth, on the palatal incline of the buccal cusp, using a rounded steel loading tip measuring 1.3 mm in diameter in a servohydraulic material test system (MTS model 801, MTS Corporation, Eden Prairie, MN, USA). Noncycled teeth were subjected to a

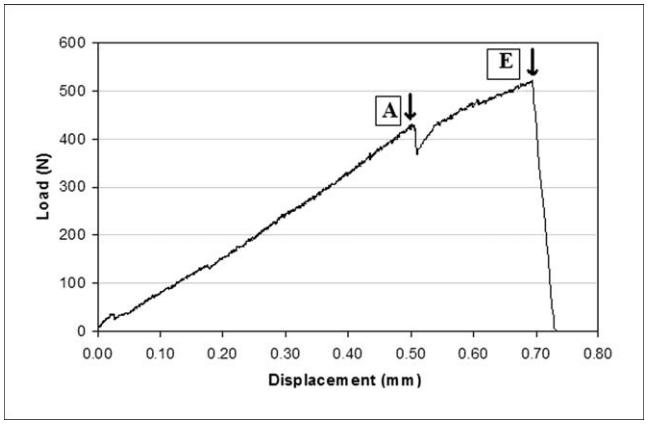

Figure 2. *Typical load displacement curve showing the initial failure (A) and the final fracture (E) loads.*

ramped load at a rate of 0.5 mm/min until fracture. Fatigue-tested subgroups were subjected to cyclic loading, with a preconditioning load of 100 N (5000 cycles, 5 Hz), followed by stepped loads of 200 N and 250 N and higher loads in 50-N increments, as needed, for 30,000 cycles each (3-5 Hz) until fracture. Fracture load (N), number of cycles, and fracture patterns were recorded. Fracture strengths were compared statistically using two-way analysis of variance with Bonferroni test for multiple comparisons.

Mode of failure (adhesive, cohesive) was investigated by light microscopy at 20× magnification. For purposes of data analysis, the failure load and fracture load were analyzed separately. Failure load was defined as the load at which debonding occurred; if a separate debonding event did not occur, then the load at fracture was considered to be the same as the failure load. Fracture load was defined as the load at which complete cusp fracture occurred. For teeth subjected to cyclic loading, the number of cycles to failure and fracture were also recorded separately.

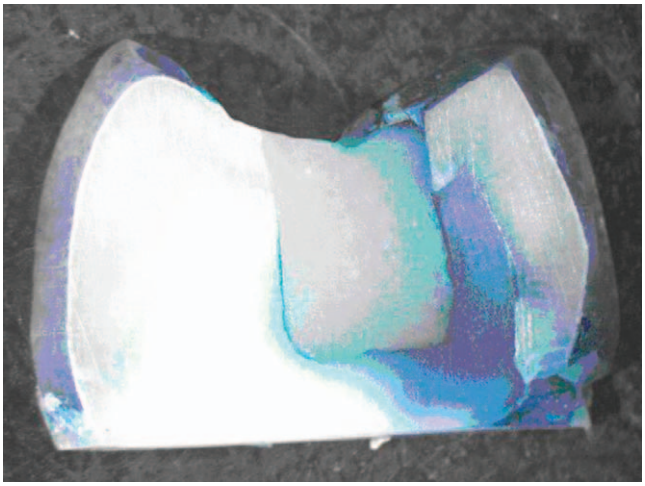

Figure 3. *Light micrograph (20× magnification) of a section of a tooth restored with resin composite and showing debonding without fracture. The tooth was immersed in methylene blue stain before sectioning and shows dye penetration along the interface between the buccal cusp and restoration, extending from the buccal occlusal surface across the base of the cavity to the palatal interface. No evidence of a fracture of the buccal cusp is present.*

RESULTS

Debonding Failure vs Fracture

A common finding in the study was the occurrence of an initial sudden debonding at the tooth-restoration interface, accompanied by an audible, sharp cracking sound that could also be detected in the load-displacement curve by a sudden drop in load (Figure 2), before the tooth underwent actual fracture. This phenomenon was observed in most teeth (31/48) undergoing both static and cyclic loading (Table 1). Debonding at either the buccal or palatal interface of the restoration was confirmed in additional teeth by stopping the load immediately after the sound was heard; staining the tooth with methylene blue and examining both the restoration margins and cross sections of the tooth demonstrated that marginal failure had occurred without cuspal fracture (Figure 3). A similar result was observed with silver staining

Table 1: *Pattern of Failure in the Three Restoration Groups in Response to Static and Fatigue Loading*

		Separate Debonding Event?		Fracture Site[a]		
Restoration Type	**Load Pattern**	**Yes**	**No**	**Buccal**	**Palatal**	**Other**
Composite	Static	5	3	6	1	1
	Fatigue	5	3	6	0	2
GIC liner	Static	6	2	7	1	0
	Fatigue	6	2	2	5	1
Open laminate	Static	3	5	4	4	0
	Fatigue	6	2	3	4	1

[a] *Fracture site refers to the tooth-restoration interface at which debonding occurred, accompanied by buccal cusp fracture. "Other" refers to fracture within the buccal cusp (two teeth), root fracture (two teeth), or debonding at both buccal and palatal interfaces (one tooth).*

Table 2: *Mean Failure and Fracture Loads in Newtons (N) for the Test Groups With and Without Cyclic Fatigue*

	Ramped Loading		Cyclic Fatigue	
Group	**Failure Load ± SD**	**Fracture Load ± SD**	**Failure Load ± SD**	**Fracture Load ± SD**
Composite	390 ± 126	490 ± 78	310 ± 68	310 ± 68
GIC liner	326 ± 58	379 ± 56	260 ± 69	265 ± 65
Open laminate	330 ± 57	352 ± 53	261 ± 69	264 ± 67

Abbreviation: SD, standard deviation.

(not shown). The load at which this debonding occurred was recorded and is referred to as failure load; the frequency of its occurrence in the different groups is presented in Table 1. Most such teeth fractured soon after debonding, but some teeth survived a considerably higher load or many additional load cycles before complete cuspal fracture occurred.

Failure Load

Load at failure was significantly lower in the groups subjected to fatigue cycling than in the static loading groups ($p<0.001$; Table 2). No differences were found among the different types of restorations ($p=0.098$). The number of cycles to failure in the fatigue testing groups varied over a wide range (5800-128,012 cycles; Table 3), with no significant differences among restoration types.

Fracture Load

Fatigue cycling had a highly significant effect in reducing fracture strength compared with static loading ($p<0.001$; Table 2). The type of restoration also had a significant impact on the fracture load ($p<0.001$). Teeth restored with resin composite alone were significantly stronger ($p=0.001$) than the liner and laminate groups (fracture 490±78 N vs 379±56 N and 352±53 N, respectively, in the static loading groups, and 310±68 N vs 265±65 N and 264±67 N in the fatigue cycling groups). The number of cycles to fracture varied from 9000 to 128,012 cycles, with no significant differences among groups ($p=0.36$; Table 3).

Failure and Fracture Modes

All failures occurred by debonding at the buccal or palatal interface between the restoration and the cavity wall, except for two cases of fracture within the buccal cusp and two cases of root fractures (Table 1). In the open laminate group, debonding was evenly distributed between the buccal and palatal interfaces, while other restorations failed predominantly at the buccal interface. Fracture almost always (45/48) involved the buccal cusp, with the crack initiating at the buccal line angle of the proximal box and extending obliquely to a subcrestal fracture on the buccal root surface (Table 1).

DISCUSSION

Teeth are subjected to repetitive occlusal loading during normal function, estimated to reach loads of up to 300 N (but more typically loads of 50-60 N) and approximately 1,200,000 cycles per five years.[24-29] Thus, it is more likely that clinical restorative failures many years after restoration may be a function of fatigue rather than a single episode of high occlusal stress.[13,29] This repetitive stress is not easy to replicate in experimental studies. Fatigue testing is a lengthy procedure and is widely considered to be impractical for testing teeth and restorative materials, especially if physiological loads are used during testing.[30,31] The use of stepped loading in this study was based on established experimental protocols by previous studies of indirect and direct coronal coverage restorations.[16,21,22] Testing begins with a low preconditioning load for 5000-10,000 cycles and then proceeds with 200-N increments at a maximum of 30,000 cycles at each

Table 3: *Number of Cycles to Failure (Debonding) and Fracture in the Three Restoration Groups Subjected to Fatigue Testing*

Restoration Type	Cycles to Failure,[a] Mean (Range)	Cycles to Fracture,[b] Mean (Range)
Composite	72,898 (9000-127,085)	75,208 (18,095-127,085)
GIC liner	48,447 (6502-128,012)	48,499 (6583-128,012)
Open laminate	47,297 (5800-126,463)	52,539 (5800-127,805)

[a] *Failure was defined as debonding of the restoration (see text for greater detail).*
[b] *Fracture was defined as the actual cusp or tooth fracture.*

load until fracture occurs. The only modification in our protocol was reducing the preconditioning load to 100 N and then increasing it in 50-N increments, based on the mean fracture loads of comparable groups in a previous experiment with ramped loading.[23] The earlier studies mentioned above used stepped fatigue testing as the standard protocol for testing and did not compare the effect of fatigue on fracture strength compared to static loading.

The number of cycles varied widely among teeth within groups, and significant differences between groups could not be demonstrated. Overall, cyclic loading negatively affected the fracture strength of teeth and may be considered an essential step in evaluation of restorative materials; however, for the sake of comparison between different techniques, the ramped load approach can still be an acceptable approach. Bolhuis and others[32] looked at the effect of fatigue loading (1 million cycles vs control) on the retention of carbon fiber post–resin composite core of maxillary premolars and found it an insignificant variable compared to the effect of cement type. Jantarat and others[29] found cyclic fatigue to cause minimal cumulative cusp displacement and concluded that it may not contribute directly to cuspal damage but rather to bond failure between the restoration and the tooth. However, our results showed almost comparable results of frequency of bond failure between ramped and fatigue-tested groups, which implies that the bond yields first regardless of the type of loading.

Of interest is the fact that failure patterns were consistent in the three restoration groups regardless of the type of loading. Almost all teeth showed debonding at the tooth-restoration interface (predominantly at the buccal margin), with fracture initiating at the buccal line angle of the proximal box and extending obliquely to subcrestal fracture of the buccal cusp. The pattern of failure was identical to that reported previously,[23,33,34] although fracture patterns vary among studies depending on tooth mounting and the direction and location of occlusal loading.[7,35]

Separate debonding was a common event before fracture in the three groups with both types of loading, similar to the findings of Hatta and others[36]; its occurrence ranged from 37% in the open laminate static load group to 62% in the composite groups and up to 75% in the fatigue-cycled open laminate group and the liner groups. The fact that most teeth survived additional cycles or higher loads before actual cusp fracture implies that the restoration did not strengthen these teeth. Otherwise cuspal fracture would occur immediately after debonding at the tooth-restoration interface. This observation also raises a concern if debonding without fracture happens clinically. If the restoration fails and a gap is open (mostly unseen) well before the cusp fractures and symptoms appear, this will invite secondary caries and may negatively affect the future restorability of the tooth. Further investigations are needed to demonstrate where failure initiates and the pattern of crack propagation.

Resin composite restoration was significantly stronger than the liner and laminate groups in terms of fracture but not in terms of failure. This could be explained by the higher mechanical properties of resin composite compared to GIC[10]; however, it is worth mentioning that, in some countries, Fuji VII has been superseded by the light-cured Fuji Triage, which may have better mechanical properties. This result is in agreement with some previous findings[2,8,37] but contrary to other studies.[34,38] Differences could be related to variation in the method and point of loading, type of GIC used (conventional vs resin modified), thickness of the base, type and size of cavity preparation, and anatomical variation of teeth. Despite the ability of GIC to bond to dentin, it was not able to shift the fulcrum point for cuspal fracture to a higher level than that proposed by Hood[39]—the buccal line angle of the proximal box, and therefore did not change the fracture pattern to a more favorable one (supracrestal).

Although the use of a GIC base has been shown to be beneficial in terms of improving the proximal marginal seal of resin composite restorations, particularly the resin-modified types,[9,11,40] attention should be paid to the fact that GIC has lower mechanical strength than does resin composite, and, therefore, it should be used in minimum thickness to achieve this goal without compromising the strength.

CONCLUSION

- Resin composite restoration was significantly stronger than both the glass ionomer core and laminate techniques.
- Bond failure remains a major concern with resin composite restorations.
- Fatigue cycling had a negative effect on the fracture strength of restored teeth.

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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Enamel Wear Opposing Polished and Aged Zirconia

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Clinical Relevance

Zirconia is an esthetic material with mechanical properties similar to steel. However, when subjected to loading in the oral cavity, zirconia exhibits phase transformation that increases the surface roughness. The roughness, however, does not increase opposing enamel loss.

SUMMARY

Aging of dental zirconia roughens its surface through low temperature degradation. We hypothesized that age-related roughening of zirconia crowns may cause detrimental wear to the enamel of an opposing tooth. To test our hypothesis, we subjected artificially aged zirconia and reference specimens to simulated mastication in a wear device and measured the wear of an opposing enamel cusp. Additionally, the roughness of the pretest surfaces was measured. The zirconia specimens, artificially aged by autoclave, showed no significant increase in roughness compared to the nonaged specimens. Furthermore, no significant difference in material or opposing enamel wear between the aged and nonaged zirconia was seen. All zirconia specimens showed less material and opposing enamel wear than the enamel to enamel control or veneering porcelain specimens. Scanning electron micrographs showed relatively smooth surfaces of aged and nonaged zirconia following wear testing. The micrographs of the veneering ceramic showed sharp fractured edges and fragments of wear debris. Zirconia may be considered a wear-friendly material for restorations opposing enamel, even after simulated aging.

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INTRODUCTION

As a result of its unique microstructure, zirconia has mechanical properties superior to those of other dental ceramics.[1] It is a multiphasic ceramic, existing in three temperature-dependent crystallographic structures: monoclinic (room temperature-1170°C), tetragonal (1170°C-2370°C), and cubic (above 2370°C).[2] The tetragonal phase of zirconia

can be stabilized at oral temperatures by alloying the zirconia with an oxidizer, such as yttria.[3] Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) is metastable at oral temperatures, meaning that mechanical and thermal stimuli induce a tetragonal to monoclinic (t-m) transformation.[4] This phase transformation causes a 4.5% volume expansion in the material and presents both advantages and disadvantages for Y-TZP.[5] Following crack initiation in Y-TZP, the t-m phase-induced expansion compresses the growing crack tip in a phenomenon known as transformation toughening.[4] The t-m phase transformation can also spontaneously occur over time in a process known as low temperature degradation.[6]

Zirconia is used in dental applications for its dual esthetic and mechanical advantages. As a result of its opacity, early uses of dental zirconia were for crown cores and frameworks over which a more translucent veneering porcelain was applied. As a result of a mismatch in the coefficient of thermal expansion of the core zirconia and the veneering porcelain,[7] however, chipping of the veneering porcelain was observed. In response, monolithic, full-contour zirconia restorations were introduced.[8] An initial concern with nonveneered zirconia was wear of opposing enamel, as zirconia is over twice as hard as veneering porcelain.[9,10] Recent studies,[11,12] however, have shown that polished zirconia appears to be wear-friendly with regard to opposing tooth structure.

Although the *in vivo* wear of zirconia is a relatively new problem in dentistry, zirconia has been used in artificial hip joints since the late 1980s, and orthopedic investigators[13,14] have noted increased roughening and wear of zirconia after prolonged *in vivo* service. As zirconia ages, it undergoes low temperature degradation. This degradation has been associated with surface roughening and particle release,[15] which increase its wearing potential. The wear associated with zirconia-based artificial hip joints led to manufacturer recalls[16]; therefore, it is critical to examine the effects of zirconia aging for dental applications. Since full- contoured zirconia crowns have only been commercially available for a short period of time, it is necessary to artificially age zirconia in order to predict its long-term wear performance. Several methods exist to artificially age zirconia, including autoclaving and boiling.[16-18] In this project, zirconia was artificially aged by autoclave.

In this study, we examined polished and aged zirconia. Roughness, wear, and opposing tooth wear were compared. Enamel and veneering porcelain were used as controls. The null hypothesis was that there would be no difference in wear or opposing enamel wear between any of the tested materials.

MATERIALS AND METHODS

Specimen Preparation

Sixteen Y-TZP specimens (LAVA, 3M ESPE, St Paul, MN, USA) were surface polished in the pre-sintered (green) state with 2500-grit silicon carbide sandpaper and then sintered at 1500°C. Eight of the specimens were then polished with a 3-μm diamond disk and polishing compound as the final step and assigned to the polished group. The remaining eight specimens were heated in a dental autoclave (biomedis FVS 2 Steam Autoclave, biomedis GmbH) for five hours at 135°C and two-bar pressure. These specimens were assigned to the aged group. Specimens (n=8) of a veneering porcelain (Lava Ceram; 3M ESPE) were prepared by the manufacturer. Flat human maxillary incisors (n=8) were collected for reference enamel and mounted to expose their facial surfaces. Opposing enamel cusps (antagonists) were prepared from extracted caries-free mandibular molars. Their mesiobuccal cusps were standardized to a cone (diameter=5 mm, height=2 mm) with a diamond bur (Sintered diamond part #5014006OU; Brasseler, Savannah, GA, USA). The cusp tips were not abraded by the standardizing bur and therefore represent uncut enamel.

Wear Testing and Measurement

Wear testing was performed in the modified Alabama wear testing device. A vertical force of 10 N was applied by enamel antagonists followed by a 2-mm horizontal slide. The test was cycled at 0.66 Hz, stopping at 100,000 and 200,000 cycles. A 1:3 glycerine:distilled water solution continuously lubricated the specimens. Impressions of the enamel styli were taken with siloxane impression material (Imprint 3 Light Body; 3M ESPE) at baseline and at 200,000 and 400,000 cycles and poured with gypsum die stone (Silky-Rock; Whip Mix Corporation, Louisville, KY, USA). The stone models and wear specimens were scanned with a noncontact light profilometer (Proscan 2000; Scantron Industrial Products Ltd, Tauton, UK) at a 20 μm × 20 μm resolution. The profilometer scans at baseline-100,000 cycles and baseline-200,000 cycles were superimposed (ProForm Software; Scantron Industrial Products Ltd) and aligned to measure the volumetric loss of enamel and ceramic. Representative samples were then examined by light microsco-

Table 1: *Volumetric Wear of Enamel Antagonists and Ceramic/Enamel Substrates and Roughness of Pretest Substrates*[a]

	Volumetric Enamel Antagonist Wear, mm^3		Volumetric Ceramic/Enamel Substrate Wear, mm^3		Ceramic/Enamel Substrate Roughness (Ra), μm Ra
	100,000 Cycles	200,000 Cycles	100,000 Cycles	200,000 Cycles	Pretest
Polished zirconia	0.099 ± 0.027 A	0.177 ± 0.049 A	—	—	0.04 ± 0.01 A
Aged zirconia	0.139 ± 0.023 A	0.202 ± 0.032 A	—	—	0.10 ± 0.05 A
Veneering porcelain	0.359 ± 0.053 C	0.512 ± 0.051 C	0.28 ± 0.05 B	0.36 ± 0.06 B	0.35 ± 0.05 A
Enamel	0.237 ± 0.045 B	0.358 ± 0.075 B	0.17 ± 0.03 A	0.27 ± 0.06 A	2.37 ± 0.74 B

[a] *Similar letters represent statistically similar groups within each column.*

py (VHX600; Keyence, Itasca, IL, USA) and scanning electron microscopy (SEM) (Quanta FEG 650; FEI, Hillsboro, OR, USA). Specimens were gold-palladium sputter-coated prior to SEM. Enamel specimens became desiccated during sputter-coating and therefore could not be imaged with SEM.

Roughness Measurement

Surface roughness (Ra) of all the specimens was determined using a noncontact light profilometer (Proscan 2000). As pretest surfaces were assumed to be homogeneous, an area in the middle of each specimen was selected for testing. A 0.7-μm length was measured with a 0.8- mm cutoff length and a 40 surface filter number selected for polished and aged zirconia and a 2.5-mm cutoff length and a 125 surface filter number selected for all other groups (based on ISO 4288-1996).

Statistical Analysis

A repeated-measures general linear model was used to determine significant differences between the paired groups of 100,000 and 200,000 cycles (α=0.05) and the differences between material groups (α=0.05). Post hoc analyses among group means were conducted using a Tukey test (α=0.05).

RESULTS

The volumetric wear of the opposing enamel specimens, as well as the ceramic and enamel specimens, are both presented in Table 1. The samples and opposing enamel had significantly more wear after 200,000 cycles than after 100,000 cycles ($p<0.01$). The enamel opposing aged and nonaged zirconia showed statistically similar wear. The enamel-enamel group showed significantly more wear than both of the zirconia groups, but the greatest wear was seen on enamel opposing veneering porcelain ($p<0.05$).

At both 100,000 and 200,000 cycles, no detectable wear was measured on either the polished or aged zirconia. The veneering porcelain showed significantly more wear than the enamel control ($p<0.05$). Light micrographs of the ceramic and enamel specimens can be seen in Figure 1. No detectable scratches or cracks can be seen on the surface of the polished or aged zirconia samples. The veneering porcelain and enamel exhibited a rough wear scar.

SEM of the polished and aged zirconia showed relatively smooth surfaces of ceramic at an original magnification of 5000× (Figure 2) and only fine scratches from polishing could be observed. SEM images of the veneering porcelain showed sharp fractured edges of the porcelain in the wear scar (Figure 2). Additionally, fragments of the worn material could be seen on the specimen surface.

Roughness values are presented in Table 1. Both zirconia groups and the veneering porcelain had statistically similar roughness values. The enamel group was significantly rougher than all other groups ($p<0.05$).

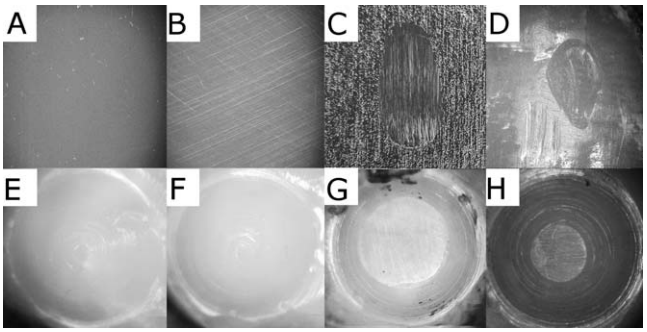

Figure 1. *Light micrographs of (A) polished zirconia, (B) aged zirconia, (C) veneering porcelain, (D) incisor enamel, (E) enamel opposing polished zirconia, (F) enamel opposing aged zirconia, (G) enamel opposing veneering porcelain, and (H) enamel opposing enamel.*

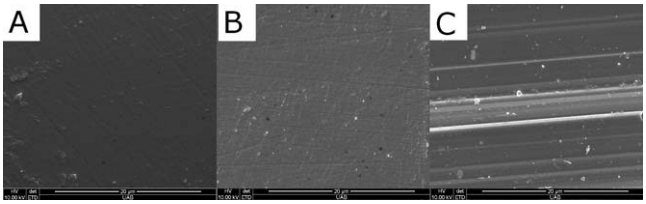

Figure 2. *SEM micrographs of (A) polished zirconia, (B) aged zirconia, and (C) veneering porcelain.*

DISCUSSION

Aged zirconia demonstrated statistically similar amounts of wear and produced similar amounts of opposing enamel wear when compared to nonaged, polished zirconia. Both zirconia groups showed less wear and opposing enamel wear than the control enamel and veneering porcelain groups. Therefore, the null hypothesis is rejected. Within the limitations of this study, the long- term wear of zirconia and its opposing enamel should be as clinically acceptable as that of veneering porcelain or natural enamel.

The three basic substrates in this study (veneering porcelain, zirconia, and enamel) all have different microstructures and methods of wear. The veneering porcelain used in this study, Lava Ceram (3M ESPE), is a feldspathic porcelain designed specifically for veneering zirconia frameworks. It is composed of a crystalline leucite phase surrounded by an amorphous glassy matrix.[20] Presumably, Lava Ceram would have a low concentration of leucite phase (5%-10%) to lower its coefficient of thermal expansion to match zirconia.[21] This crystalline leucite phase contributes to its mechanism of wear in two ways. The hard leucite crystals are more abrasive than the surrounding glass and can more easily damage opposing enamel. The leucite crystals also strengthen the porcelain through dispersion strengthening and help prevent fracture.[20,22] The porcelain material used in this study is predominantly composed of glass, and therefore it is susceptible to fracture. The SEM image of the veneering porcelain (Figure 2) shows the sharp edges of porcelain that have been fractured through the wear process. Fragments of wear debris are observed on the surface of the specimen. We theorize that the sharp fractured edges of the worn porcelain and the hard fragments of wear debris caused abrasive damage to the opposing tooth structure. The wear process may have been initiated by the initial roughness of the porcelain (Ra=0.35±0.05 μm). A previous study showed that hydroxyapatite (HA) wear increased by a 20-fold measure as a counter–surface roughness was increased from 0.014 to 0.649 μm Ra.[23] Unlike veneering porcelain, zirconia is a polycrystalline ceramic with no glass content.[20] Its crystalline microstructure along with its transformation toughening ability make zirconia very resistant to fracture.[9] The SEM image of polished zirconia (Figure 2) shows no signs of scratching or cracking after 200,000 cycles of wear. We theorize that the smooth surface of worn zirconia prevented abrasive wear of the opposing enamel. Aging zirconia roughens its surface through the growth of transforming monoclinic phases and the corresponding surface relief.[19,24] This study is in agreement with other studies,[24,25] that aging zirconia increases its surface roughness (although not by a statistically significant amount); however, no increase in opposing enamel wear was noted. The SEM image of the aged zirconia (Figure 2) demonstrates a similar surface smoothness to nonaged zirconia. A study by Liu and Xue[26] found that increasing the normal load applied to zirconia during sliding wear above 20 N altered the mechanism of wear from plastic deformation to microcracking. The shift in wear mechanism led to increased wear.

Enamel is composed of HA crystals embedded in an organic matrix. The orientation of these crystals divides enamel into structures known as rods.[27] Analysis of enamel subjected to sliding wear reveals that it fails by microcracking.[28] Pure fluorapatite (similar to HA) wears by brittle fracture.[29] The arrangement of HA into rod structures in enamel can hinder the propagation of cracks by redirecting them.[28] The light micrograph of the worn enamel demonstrates a rough wear scar. We speculate that wear of the contacting enamel surfaces was initiated by the relatively rough enamel surface (Ra=2.37±0.74 μm) and potentiated by fracturing of opposing enamel asperities.

Enamel is an anisotropic material, as its mechanical properties are dependent on the orientation of its rods.[28] In this study, the incisors used as enamel specimens for the enamel- enamel group were oriented with their facial surfaces in contact with the occlusal surface of the antagonist tooth. We chose this orientation with the assumption that enamel rods are aligned perpendicular to the surface of the tooth. A study by Fernandes and Chevitarese,[30] however, indicated that rods are oriented at different directions in different parts of the tooth. Wear of tooth structure is also significantly increased when the layer of enamel is worn away to expose dentin.[28] The depth of the wear did not exceed 0.1 mm on any of the incisor specimens,

however, so we can assume that all wear occurred in the enamel.

The zirconia in this study was aged in an autoclave for five hours. Although there has not been a study to show the direct correlation between the time of aging *in vitro* and *in vivo,* a previous study[18] has shown that autoclaving will transform surface tetragonal zirconia to its monoclinic phase and roughen its surface. Additionally, retrieval studies[13,14] have shown that zirconia undergoes monoclinic transformation *in vivo* over time, leading to increased roughness. Therefore, autoclaving zirconia appears to be a reasonable method for simulating accelerated aging.

CONCLUSIONS

The results of this study are in agreement with those of earlier studies that found that zirconia causes less wear to opposing teeth and experiences less surface wear than enamel or a veneering porcelain.[12] Additionally, the results of this study indicate that age-related degradation of zirconia does not make this material more likely to induce or undergo wear. Future studies should examine wear of zirconia at forces beyond 20 N to determine if microcracking occurs.

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 Sodium Ascorbate on Dentin Bonding After Two Bleaching Techniques

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PH dos Santos • RS Alexandre

Clinical Relevance

Bleaching with either 10% carbamide peroxide or 35% hydrogen peroxide impairs the formation of the hybrid layer, resin tags, and bond strength. The use of sodium ascorbate following bleaching diminishes this adverse effect in the case of 10% carbamide peroxide but not so when 35% hydrogen peroxide is used as the bleaching agent.

SUMMARY

The purpose of this study was to analyze the influence of 10% sodium ascorbate (SA) on the hybrid layer, resin tag length, and bond strength to dentin after bleaching. Six groups were tested: G C, control; G SA, sodium ascorbate (SA) + restoration; G CP, bleaching with carbamide peroxide (CP) + restoration; G CP+SA, bleaching with CP + SA+ restoration; G HP, bleaching with 35% hydrogen peroxide (HP) + restoration; and G HP+SA, HP + SA + restoration. After dental bleaching, the dentin was exposed and the antioxidant solution was applied to groups G SA, G CP+SA, and G HP+SA, before bonding procedures. The teeth were sectioned in the mesiodistal direction. One section was decalcified, and the specimens were embedded in paraffin and sectioned in the longitudinal direction with a thickness of 6 μm. Fifteen slices of each specimen were selected according to a systematic sample of slices with an interval proportional to the total number of slices obtained for each tooth. The specimens were stained using the Brown & Brenn method, and an optic microscope was used to analyze the hybrid layer thickness and resin tag length. The remaining tooth segment was sectioned into stick-shaped specimens and used for microtensile bond strength testing (0.5 mm/min). Statistical analysis was performed using two-way analysis of variance and Fisher test. The results for hybrid layer +

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tag formation (in micrometers) were G C, 13.27 Aa; G SA, 11.85 Ba; G CP, 6.84 Bb; G CP+SA, 9.02 Ab; G HP, 7.28 Bb; and G HP+SA, 9.22 Ab; bond strength results (in MPa) were G C, 49.5 Aa; G SA, 51.7 Aa; G CP, 37.16 Bb; G CP+SA, 47.69 Aa; G HP, 32.39 Ab; and G HP+SA, 39.67 Ab. Tooth bleaching with CP or HP impairs the formation of the hybrid layer and resin tags and reduces the microtensile bond strength. Statistically, the use of SA significantly increases the hybrid layer thickness and resin tag length. The microtensile bond strength values for carbamide peroxide increased, but the microtensile bond strength for hydrogen peroxide was not affected.

INTRODUCTION

In general, the techniques for tooth bleaching are simple and generate positive esthetic results when the manufacturer's directions are followed. In addition to the traditional at-home bleaching technique with carbamide peroxide products in bleaching trays, hydrogen peroxide at high concentration has produced faster results for those patients who cannot or do not want to wear the trays.[1,2]

It is suggested that esthetic restorations should be replaced after tooth bleaching, since color alteration affects tooth structure but not restorative materials.[3,4] A 7-day waiting period following bleaching is recommended before replacing adhesive restorations because bleaching products adversely affect the bonding of resin materials to the tooth and overcome low bond strength values to bleached dental surfaces, which may negatively influence the clinical performance of restorations.[2,4-7] Moreover, there are other reasons for delaying restorative procedures, such as reducing postoperative sensitivity and to allow time for color rebound, which may alter esthetic results.[8-10]

It is worth noting that most of the bonding studies have been conducted with resins placed primarily in enamel,[1,7,11,12] while some recent studies evaluated the effect of tooth bleaching on bonding to dentin.[2,13-17] Most restorative procedures use enamel and dentin as substrates for adhesion.

In the traditional adhesive technique with prior acid etching, the smear layer and smear plug are removed and superficial demineralization of dentin occurs, exposing the collagen fibers. The penetration of the adhesive system into this demineralized structure and in the tubules allows formation of a hybridized region (resin tags + hybrid layer), which restores the structural resistance of the etched region[18] and prevents the occurrence of microleakage.[19]

Although some authors have indicated that a thin hybrid layer can have a performance similar to a thicker one,[20] other studies[21,22] have reported that a thin hybrid layer and a reduced penetration of the adhesive systems in dentin could result in lower bond strength values.

Many studies consider a 10% sodium ascorbate (SA) solution to be an effective choice to improve dentin bonding and defend the use of SA as the most efficient agent for neutralizing the oxidizing effects of bleaching agents. Its use may also increase the strength of the dentin bond.[11,13,14,23-25] The antioxidant type, concentration, form, and duration of application have been considered important factors for improving bonding after bleaching treatment.[15-17,24,25]

The reducing capacity or ability to neutralize and reverse the oxidizing effects of hydrogen peroxide in biological systems has already been reported.[26,27] Some authors have suggested that the use of SA after bleaching can reverse the reduced adhesion between adhesive materials and dental tissue.[11,13,17,24,25]

However, since there are no significant reports emphasizing the effect of antioxidant agents on the formation of the hybrid layer and resin tags, laboratory studies are necessary to assess the effect of bleaching agents and antioxidants on the micromechanical interaction occurring in dentin. The purpose of this current study was to evaluate the influence of 10% SA solution following two bleaching techniques on the bond strength in dentin. The microtensile test was used for evaluation.

The null hypothesis tested was that the use of SA does not influence the hybridization depth or the microtensile bond strength in dentin after bleaching.

MATERIALS AND METHODS

This study investigated the influence of 10% SA on the hybrid layer, resin tag length, and bond strength to dentin after dental bleaching with two different materials (Table 1).

Thirty-six human premolars without any cracks and that were extracted for other purposes were divided into six experimental groups (n=6) according to the bleaching procedures and the use of antioxidant. The teeth were cleaned with manual periodontal curettes (Duflex Ltda, Rio de Janeiro, Rio de

Table 1: *Composition and Manufacturers of Products*

	Material	Composition	Manufacturer	Lot	Mode of Application
Composite resin	Z-250	UDMA: urethane dimethacrylate; Bis-EMA: bisphenol A polyethylene glycol diether dimethacrulate; TEGDMA: tri-ethylene glycol dimethacrylate; inorganic particles	3M/ESPE, St Paul, MN, USA	6CR	Two 2.0-mm-thick flat composite resin increments
Etching agent	Scotch Etchant	35% Phosphoric acid gel	3M/ESPE, St Paul, MN, USA	5EX	15 s
Adhesive system	Adper Scotchbond Multi Purpose	Primer: HEMA, polyalkenoic acid copolymer; adhesive: Bis-GMA, HEMA, photoinitiators	3M/ESPE, St Paul, MN, USA	Primer: 6BC, adhesive: 6PL	According to manufacturer's instructions
Bleaching products	10% Whiteness standard	Carbamide peroxide, carbopol, potassium ions, glycol, high content of water, sodium fluoride	FGM – Dental Products, Joinvile, Brazil	061008	Four hours per day for 14 days
	Whiteness HP Maxx	35% Hydrogen peroxide, thickening stain's mixture, glycol, and deionized water	FGM – Dental Products, Joinvile, Brazil	270206	Four sessions, one session per week, three 15-minute applications per session
Antioxidant	10% Sodium ascorbate	10% Sodium ascorbate and osmolality water	Farmácia Aphoticário, Araçatuba, Brazil	No batch number	10-minute application before bonding procedures

Abbreviations: Bis-GMA, bisphenol-A-glycidyl dimethacrylate; HEMA, 2-hydroxyethyl methacrylate.

Janeiro, Brazil) using distilled water to remove periodontal tissues, washed using pumice and water in a rubber cup, coupled to a slow-speed hand piece (Dabi Atlante, Ribeirão Preto, SP, Brazil), and rinsed and stored in 0.1% neutral thymol solution at room temperature until the beginning of the research.

The current study design was analyzed and approved by the Institutional Review Board for Investigations (2009-2384). Throughout the study, specimens from all study groups were stored in artificial saliva at 37°C.

In groups carbamide peroxide (CP) and CP+SA, a commercial 10% CP at-home bleaching gel (Whiteness Standard, FGM Produtos Odontológicos Ltda, Joinville, SC, Brazil) was applied to the buccal and occlusal surfaces (0.06 mL per tooth) using a graduated 1-mL syringe for four hours per day, according to the manufacturer's instructions. The specimens were thoroughly rinsed with air-water spray and stored in artificial saliva (pH=6.7).

The specimens from groups hydrogen peroxide (HP) and HP+SA were bleached using 35% hydrogen peroxide (Whiteness HP Maxx, FGM Produtos Odontológicos Ltda) applied to the buccal and occlusal surface in a layer of about 1.0 mm in thickness (0.06 mL). After one minute, the product was exposed to a quartz-tungsten-halogen light (Ultralux, Dabi Atlante) with a light output of 450 mW/cm^2 for 20 seconds, according to the manufacturer's instruction. The product remained on the dental surface for 15 minutes with no light source. Four bleaching sessions were performed, and three applications of the bleaching product were made during each session. After each bleaching sessions, the specimens were stored in artificial saliva until the next session (seven days later).

After performing the bleaching procedures, the occlusal surfaces of all specimens were abraded with 600-grit abrasive paper disks (Carbimet Paper Disks, Buehler, Lake Bluff, IL, USA) in the polisher Aropol E (Arotec Industria e Comércio Ltda) at a constant speed and under irrigation until dentin exposure. The specimens from groups CP+SA and HP+SA, bleached with 10% CP and 35% HP, respectively, had the SA antioxidant solution (Farmácia Aphoticário, Araçatuba, SP, Brazil) applied on the dentin surface for 10 minutes at 1 mL/min. The specimens were then rinsed with distilled water for 30 seconds, and bonding procedures were performed immediately after SA application. For that purpose, the exposed dentin substrate was rinsed, dried, and etched with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE, St Paul, MN, USA) for 15

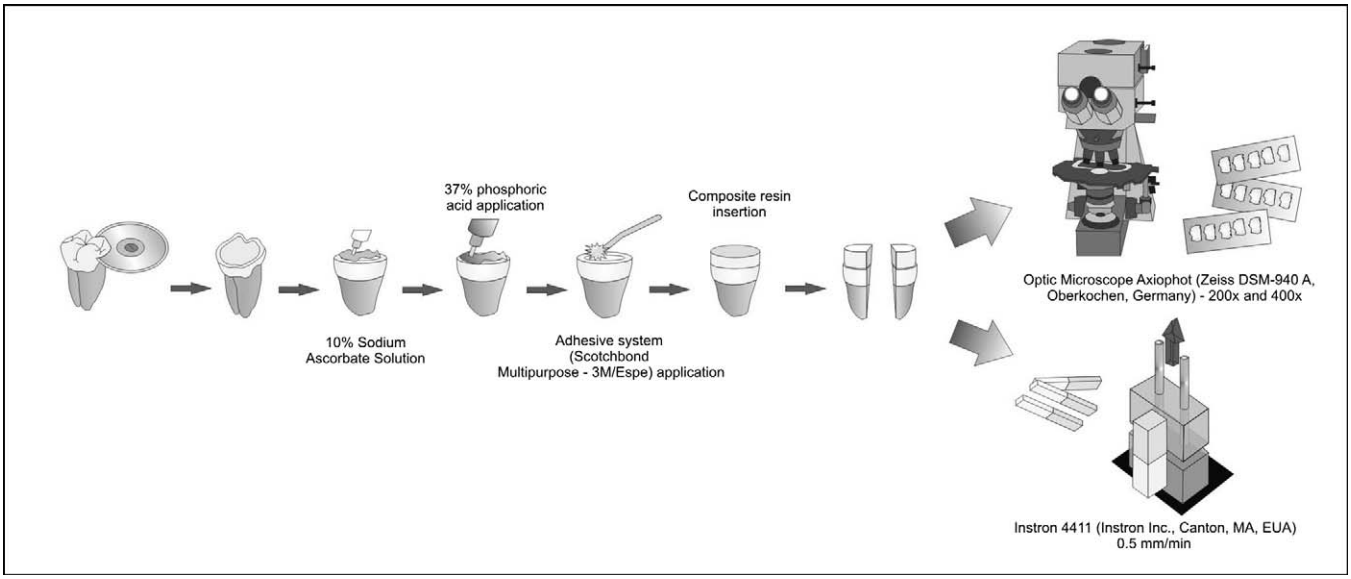

Figure 1. *Methodology scheme of the teeth sectioning into two equal parts used for microtensile test and hybrid layer and tag formation analysis.*

seconds. Then, the substrate was rinsed again for 10 seconds and dried with compressed air spray with the dentin protected by a small cotton ball to maintain the humidity of the tissue. A layer of the two-step adhesive system primer (Adper Scotchbond Multi-Purpose, 3M/ESPE) was applied on the dentin followed by the application of a layer of the hydrophobic component. Light polymerization was carried out with a halogen light–curing unit for 10 seconds at a light output of 450 mW/cm^2. The composite resin (Filtek Z250, 3M ESPE), shade A2, was inserted using a Thompson spatula and light polymerized for 40 seconds.

In group SA, 10% SA antioxidant solution (Table 1) was applied as described for groups CP+SA and HP+SA, after dentin exposure. Afterward, bonding procedures were performed.

Group C was the control, and bonding procedures were performed after dentin exposure with no previous treatments.

After the restorative procedures, all of the specimens were sectioned into two equal parts, in the mesiodistal direction, using the metallographic cutter ISOMET 2000 (Buehler) under water cooling and at a speed of 800 rpm and static load of 160 g (Figure 1). One section was decalcified over three months in a solution containing 50% formic acid and 20% sodium citrate. After demineralization, the specimens were immersed in alcohol solutions at different concentrations for dehydration. Immediately after this procedure, the specimens were immersed in xylol for one hour. The restorations were carefully removed, and the specimens were embedded in paraffin and sectioned in the longitudinal direction with a slice thickness of 6 μm. Fifteen slices of each specimen were selected according to a systematic sample of slices with an interval proportional to the total number of slices obtained for each tooth.

The selected slides were stained using the Brown & Brenn method and analyzed with an optical light microscope (Axiophot, Carl Zeiss Company, Oberkochen, Germany). The hybrid layer thickness and resin tag length were analyzed with the micrometric ocular piece 40/075.

Each slice generated three images at 400× of the mesial, middle, and distal thirds. In each visual field, the hybrid layer + resin tags were measured at five different areas using the Axiovision Software Rel. 4.6 (Carl Zeiss Company), as demonstrated in Figure 2.

The other tooth section was used for microtensile bond strength testing. A metallographic cutter was used under water cooling at a speed of 800 rpm and static load of 160 g to obtain stick-shaped specimens from the central region of each tooth, totaling 30 specimens per group.

These specimens were fixed to the Universal Test Machine Instron Model 4411 (Instron Inc, Canton, MA, USA), using cyanoacrylate adhesive (Super Bonder, Henkel Ltda, Itapevi, São Paulo, Brazil) and tested with a 50N load cell at a speed of 0.5 mm/min until failure. The bond strength values of the sticks from the same tooth were averaged, and only the bond strength values of the six teeth from each

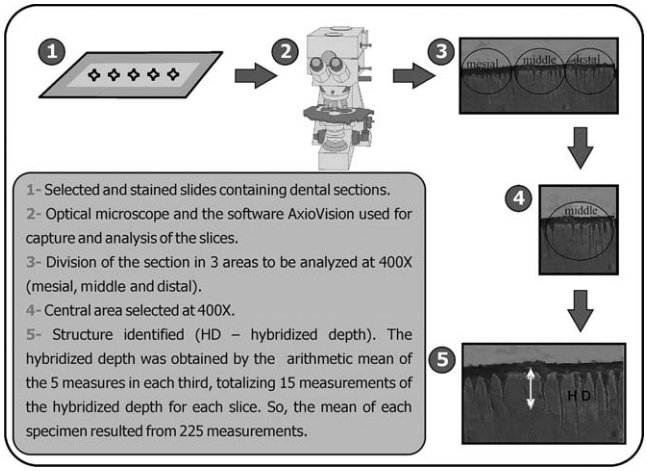

Figure 2. *Methodological sequence used to obtain the hybrid layer thickness and resin tags length.*

experimental condition were analyzed statistically. Premature failures were not considered in the data analysis.

The results obtained for hybrid layer + resin tags and microtensile bond strength were submitted to two-way analysis of variance (ANOVA) and Fisher PLSD test, at a level of significance of 5%, to verify the effect of the treatments on dentin bonding.

The fracture pattern was analyzed in the stereoscopic Stemi SV 11 (DSM-940 A, Carl Zeiss Company) at 30× magnification. To reveal the fracture area of each specimen more clearly, the revealing solution proposed by Ohkubo and others in 1982[28] was applied on the fractured region, which stained the resin-free dental structure. Images were analyzed using Image J Software (Image Processing and Analysis in Java, NIH, Bethesda, MD, USA), and the fracture patterns were determined.

RESULTS

Means of hybrid layer + resin tags (μm) and microtensile bond strength are summarized in Tables 2 and 3. ANOVA showed that significant differences were observed among the groups for both analyzed factors ($p<0.05$; Tables 4 and 5).

Specimens from G C showed greater resin tag length and hybrid layer thickness, suggesting a continuous and frequent micromechanical interaction (Figure 3). When SA was used before the restoration procedures and without bleaching treatment (group SA), the hybrid layer + resin tags were thinner than the control group ($p=0.0038$). The bleached specimens from groups CP and HP showed the lowest adhesive penetration means, presenting unusual, fewer, and shorter resin tags and a thinner hybrid layer; no significant difference was found between these groups ($p=0.335$). It was also verified that SA resulted in an increase in resin tag length and hybrid layer thickness for both CP+SA (Figure 4) and HP+SA (Figure 5).

For the microtensile bond strengths, group C and group SA showed statistically similar values ($p=0.59$). In the CP group, the SA application increased the values of microtensile bond strength, but the SA application was not effective for the 35% HP bleached teeth (Tables 3 and 5).

Fracture pattern analysis showed a prevalence of adhesive failures. Groups CP and HP+SA presented a high incidence of cohesive failures in dentin, while cohesive failures in resin were frequent in groups C and SA (Figure 6).

DISCUSSION

At-home bleaching techniques have been widely used to obtain improved esthetic results.[3] Many studies have been conducted to clarify the interaction of bleaching, its effect on hard tissues, and its influence on bonding adhesive restorative materials to the tooth structure.[12]

In recent studies, microleakage, a decrease in bonding strength, and low micromechanical interaction were associated with bleaching procedures.[6,29,30] These conditions were augmented when the in-office bleaching technique, which uses bleach at a higher concentration, was performed.[31,32] As a result, a 24-hour to one-month waiting period after bleaching has been recommended before performing restorative procedures.[2,6,29]

Table 2: *Length of Hybrid Layer + Resin Tags (μm) for All of the Studied Groups (Means ± Standard Deviation)*[a]

	Group C	Group CP	Group HP
No SA	13.27 ± 0.94 A a	6.84 ± 0.41 B b	7.28 ± 0.36 B b
+SA	11.85 ± 0.76 B a	9.02 ± 0.84 A b	9.22 ± 1.10 A b

Abbreviations: C, control; CP, carbamide peroxide; HP, hydrogen peroxide; SA, sodium ascorbate.
[a] *Means followed by different letters (capital letter in vertical; lower case in horizontal) represent a significant difference according to analysis of variance and Fisher test,* $p \leq 0.05$.

Table 3: *Values of Microtensile Bond Strength (MPa) for All of the Studied Groups (Means ± Standard Deviation)*[a]

	Group C	Group CP	Group HP
No SA	49.54 ± 8.09 A a	37.16 ± 3.10 B b	32.39 ± 11.73 A b
+SA	51.73 ± 5.52 A a	47.69 ± 6.47 A a	39.67 ± 7.27 A b

Abbreviations: C, control; CP, carbamide peroxide; HP, hydrogen peroxide; SA, sodium ascorbate.
[a] *Means followed by different letters (capital letter in vertical; lower case in horizontal) represent significant difference according to analysis of variance and Fisher test, p≤0.05.*

Table 4 *Analysis of Variance Table for Hybrid Layer + Resin Tags*

	df	Sum of Squares	Mean Square	*F*-Value	*p*-Value	Lambda	Power
Bleaching agent	2	160.661	80.330	131.124	<0.0001	262.248	1.000
Antioxidant agent	1	7.326	7.326	11.958	0.0017	11.958	0.934
Bleaching agent/antioxidant agent	2	24.255	12.128	19.796	<0.0001	39.592	1.000
Residual	30	18.379	.613				

The decrease in bond strength and reduction of the adhesive system interaction in bleached teeth are attributed to the presence of residual oxygen.[13,17,33,34] This highly reactive chemical element eliminates pigmentation but also reacts with the free radicals of the resin materials, which inhibits polymerization and generates polymers with reduced mechanical properties.[25,33,35,36] Some studies in dental enamel suggest that the amount of residual oxygen is proportional to the reduction in bonding and the reduced length and frequency of resin tags in the bleached substrate.[6,29] Data found in the current study also showed a reduced hybrid layer and resin tag formation. This result may be related to mechanical modifications in peritubular and intertubular dentin produced by bleaching agents that could lead to biomechanical alterations, but there is no evidence to substantiate this.[30,37]

Histochemical studies have shown reduced levels of sulfur in samples bleached with HP or CP. Sulfur is a component of proteoglycans (chondroitin sulfate and keratan sulfate), and changes in their levels indicate damage to the organic dentin matrix.[38-40]

These changes in proteoglycans might interfere in the maintenance of interfibrillar spaces,[41] which may have compromised diffusion of the adhesive into the collagen network, potentially reducing the bond strength in this current study.[42,43]

The specimens from group C (Figure 3) presented a thick hybrid layer + resin tags and also high microtensile bond strength values. The bleached groups (G CP and G HP), which presented similar results to those from previous studies, demonstrated the deleterious effects of the oxygen released by the bleaching agents.[5,13,29,35]

For those groups that received the solution of 10% SA, the group SA exhibited a reduced hybrid layer thickness + resin tag length (11.85 μm) and was considered as a control group. Although many studies report the advantages of using this antioxidant substance with bleached teeth,[11,13,24] few studies considered the use of ascorbate with unbleached teeth.[13,44] In the current study, the use of SA alone led to limited resin tags and hybrid layer when compared with the control group, although their formations remained continuous and homogeneous across the adhesive interface. Although the hybrid layer + resin tag thickness decreased in the SA group when compared with the control group, the microtensile bond strength values were similar. This difference might not be clinically significant, since the bond strength was satisfactory for both groups.

The use of SA immediately after bleaching (groups CP+SA and HP+SA) increased the hybrid layer and resin tag length when compared with groups CP and HP (Figures 4 and 5), but the effect was not as great

Table 5 *Analysis of Variance Table for Bond Strength*

	df	Sum of Squares	Mean Square	*F*-Value	*p*-Value	Lambda	Power
Bleaching agent	2	1287.886	643.943	11.423	0.0002	22.846	0.993
Antioxidant agent	1	400.067	400.067	7.097	0.0123	7.097	0.738
Bleaching agent/antioxidant agent	2	105.963	52.982	.940	0.4019	1.880	0.191
Residual	30	1691.203	56.373				

Figure 3. *Photomicrograph (group C) showing a continuous hybrid layer and frequent resin tags formation analyzed by common optical microscopy (400× magnification).*

as that from group C. Nevertheless, the hybrid layer and resin tag formation were homogeneous and continuous, especially for group CP+SA.[13,25,30] Moreover, although some authors[20] reported that hybrid layer depth is not usually related to bond strength, higher bond strength values were also verified in this experimental group.

However, in the hydrogen peroxide group (HP+SA), the bond strength values were not improved when the bleaching agent was followed by an application of SA. This may be explained by the use of highly concentrated products[45] and suggests that the use of SA was not sufficient to improve the resin tag and hybrid layer formation. It has been demonstrated that, as the duration of application for the antioxidant increases, the bond strength of the composite to dentin tissue also increases.[24]

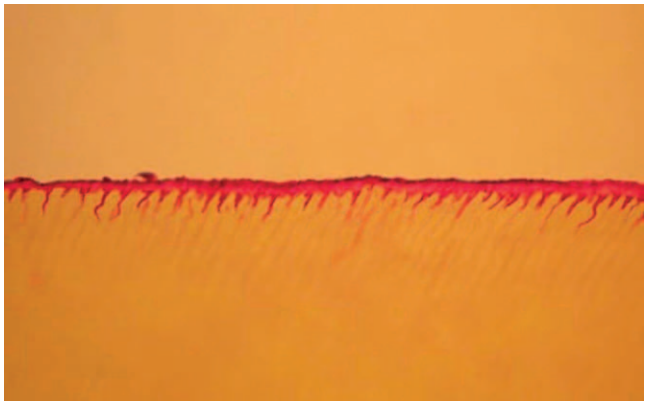

Figure 4. *Photomicrograph (group CP+SA) analyzed by common optical microscopy (400× magnification), showing a thick continuous hybrid layer and frequent but short resin tags formation.*

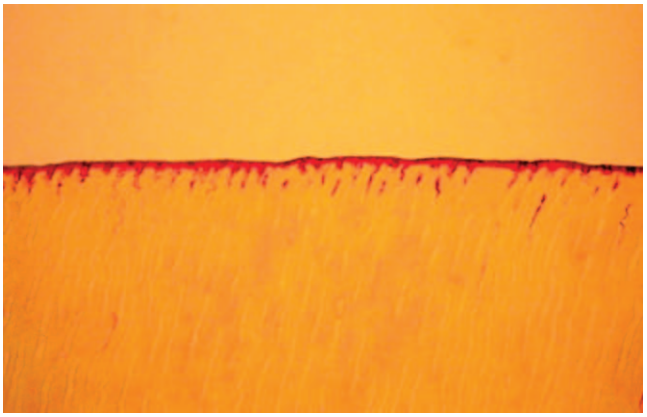

Figure 5. *Photomicrograph (group HP+SA) analyzed by common optical microscopy (400× magnification), showing a thin and continuous hybrid layer but sparse and short resin tags formation.*

According to the results of the present study, the use of SA improved adhesion in bleached teeth, suggesting that the antioxidant action may reverse (partially or fully) the damaging effects of the bleaching products.[11,13,17,24,25] It is possible that by restoring the reducing potential (redox) of the oxidized substrate, SA limits premature polymerization of the free radicals of the adhesive and therefore cancels (reverses) the effects of the impaired bonding on the bleached dentin.[13,34]

Additional studies are required to further explain the mechanism of this reversal process, its role on bonding, and the possible beneficial effects of its systemic application. It is important to suggest additional studies comparing the effect of different antioxidant solutions considering the instability of these compounds,[46] which limit their use to only a few hours after manipulation. The clinical implica-

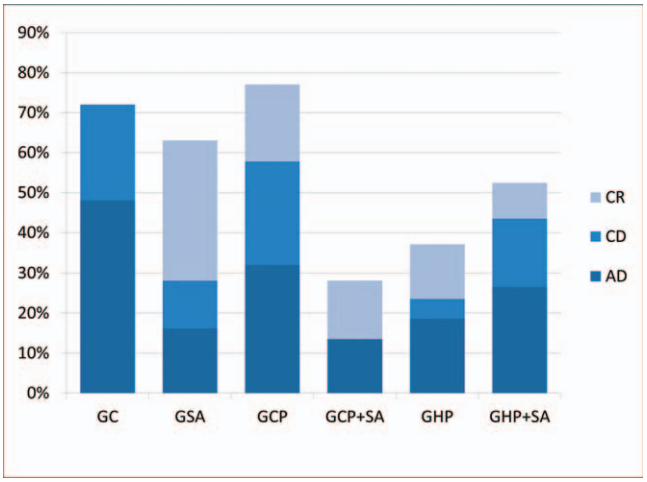

Figure 6. *Fracture pattern for the study groups. Cohesive in resin (CR); cohesive in dentin (CD); adhesive (AD).*

tion of this present study is that the application of an antioxidant can shorten the time period spent in the clinic by both dentists and patients to allow restorative procedures to be performed after bleaching treatments.[16]

Therefore, the current results do not support full acceptance of the null hypothesis, that the use of SA does not influence the hybrid layer and resin tag formation and the microtensile bond strength in dentin after bleaching.

CONCLUSIONS

Based on the present results, dental bleaching using 10% CP or 35% HP is damaging to resin tag and hybrid layer formation. When SA was applied following bleaching with CP, a better hybrid layer thickness and resin tag length developed. In this case, there was an improvement in the microtensile bond strength values. This was not the case when SA was applied following bleaching with 35% HP.

Acknowledgements

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Conflict of Interest

The authors 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 Simulated Tooth Temperature on the Degree of Conversion of Self-adhesive Resin Cements Exposed to Different Curing Conditions

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Clinical Relevance

Self-adhesive resin cements present a higher degree of conversion and faster polymerization kinetics when exposed to tooth temperature rather than to room temperature. Clinicians may expect differences in product setting time and some physical properties compared with what manufacturers report.

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SUMMARY

Objectives: This study evaluated the degree of conversion (DC) of two commercial, self-adhesive resin cements (SARCs) using Fourier transform infrared analysis (FTIR) polymerized at simulated prepared tooth surface temperatures and under different curing conditions.

Materials and Methods: RelyX U100 (U100, 3M ESPE) and Maxcem Elite (MX, Kerr Corporation) were mixed at 25°C and applied to the surface of a horizontal attenuated total reflectance (ATR) unit, which was near room temperature (RT, control) (25°C) or heated to simulate prepared tooth surface temperatures (28°C and 32°C) and then attached to an infrared spectrometer. The products were polymerized using one of three conditions: direct light exposure through a glass slide (DLE), exposure through a 1.5-mm thick ceramic disc overlay

(CO) (A2 shade, IPS e.max, Ivoclar Vivadent), or self-curing (SC). FTIR spectra were recorded for 12 minutes (1 spectrum/s, 16 scans/spectrum, resolution 4 cm^{-1}) immediately after application to the ATR. The DC was calculated using standard techniques of observing changes in aliphatic-to-aromatic peak ratios before and 12 minutes after curing, as well as during each 1-second interval. DC data (n=7) were analyzed by two-way analysis of variance and Tukey's post-hoc test (p=0.05).

Results: Both simulated tooth temperatures significantly increased DC in all groups of MX and in the CO and SC groups of U100 compared with the RT control. For MX, the self-cure groups exposed to tooth temperatures showed DC values similar to those of the CO groups. For U100, the CO groups showed higher DC values than SC groups regardless of temperature. Time-based conversion profiles ranged according to product, temperature, and curing mode.

Conclusions: Causing SARCs to polymerize at simulated tooth temperatures increases DC of SARCs compared with room-temperature curing values, mainly in the SC mode.

INTRODUCTION

The success of an indirect restoration relies on bonding materials to ensure an optimum link between the prepared tooth and the indirect restorative material. Within this context, dual-cured resin cements have been the choice of most clinicians to cement esthetic indirect restorations. To reduce technique sensitivity due to multistep bonding procedures involved in cementation techniques, manufacturers have developed the so-called self-adhesive resin cements (SARCs). These materials were introduced in the market as a new subgroup of resin cements that require no pretreatment of tooth substrate before its application.[1,2]

Similar to conventional dual-cured resin cements, SARCs depend on optimal polymerization to exhibit clinically acceptable mechanical properties and bond strength.[3-5] Because these products use similar mechanisms to initiate polymerization compared with conventional resin cements, their polymerization mode is crucial to ensure an optimal degree of conversion.[6] In this regard, the self-curing mode of such products is generally less effective than the light-activated mode,[6] and little information is available in the literature regarding the effects of light attenuation due to the presence of an indirect restoration interposed between the light-curing unit tip and the SARC layer.[7] This issue raises some concern, however, because the presence of a 1.5-mm-thick indirect restoration can cause a severe reduction of 80% to 90% in light intensity.[8,9]

Most comparisons between curing conditions and monomer conversion of SARCs are based on *in vitro* tests performed at room temperature (approximately 25°C).[6,10-12] Conversely, SARCs are applied directly to a prepared tooth, where temperature ranges from 27.8°C to 32.0°C, depending on the tooth,[13-15] and not at 37°C, as many people assume. For instance, if SARCs are used to cement indirect restorations in anterior teeth, these products will be in contact with tooth surfaces at approximately 28.0°C, while the tooth surfaces of premolars and molars will warm SARCs to approximately 32°C.[15] Previous studies have demonstrated that increased temperatures of 50°C or 60°C result in higher monomer conversion in resin cements and resin composites, regardless of the curing condition.[16,17] Therefore, it would be reasonable to expect some effect of tooth temperature in the monomer conversion of SARCs. In this regard, a previous study demonstrated that polymerization of SARCs at 37°C develops more shrinkage stress than it does at 23°C.[18]

Only a few studies have evaluated the effects of tooth temperature on SARCs, and all of them focused on bond-strength values, electron microscopy analysis, and shrinkage stress.[18-20] Although the authors attributed high strength values to elevated degree of conversion,[18-20] there is no study available in the literature directly evaluating the degree of conversion of SARCs with increased temperature.

The purpose of this study was to evaluate the effects of tooth temperatures on the degree of conversion of dual-cured SARCs exposed to different curing conditions: direct light exposure (DLE), light exposure attenuated by an indirect ceramic restoration, and activation by self-curing components with no light exposure involved. Two research hypotheses were addressed. The first hypothesis anticipated that when a ceramic restoration attenuates or totally blocks the curing light, the degree of conversion of self-adhesive cements increases as the temperature during polymerization approaches that of the clinically prepared tooth. The second research hypothesis was that, despite temperature during polymerization, the degree of conversion value when directly light-curing the SARC will not be significantly different from that attained when exposing the specimen through a porcelain overlay or when the specimen is allowed to totally self-cure without light.

Table 1: *Brand, Composition, and Batch Number of SARCs Tested*

Product (Manufacturer)	Composition (Batch Number)
Rely X U100 (3M ESPE, St Paul, MN, USA)	Base: glass fiber, methacrylated phosphoric acid esters, dimethacrylates, silanated sílica, sodium persulfate
	Catalyst: glass fiber, dimethacrylates, silanated sílica, p-toluene sodium sulfate, calcium hydroxide (367405)
Maxcem Elite (Kerr Corporation, Orange, CT, USA)	Resin: multifunctional DMAs, GPDM, proprietary Redox initiators and photoinitiators.
	Filler: barium, fluoroaluminosilicate, fumed silica (66 wt%) (423636)

Abbreviations: GPDM, glycerophosphate dimethacrylate; DMAs, dimethacrylates.

MATERIALS AND METHODS

Specimen Preparation

Two commercially available, dual-cure SARCs were tested: Rely X U100 (U100, 3M ESPE, St Paul, MN, USA) and Maxcem Elite (MX, Kerr Corp, Danbury, CT, USA). The products were selected because of their differences in composition (Table 1); only one uses calcium hydroxide to accelerate pH increase. As the product pH may have detrimental effects on the polymerization features of a resin-based material, it was expected that tooth temperature might have a different effect on the monomer conversion of a product with another pattern of pH increase caused by calcium hydroxide. A ceramic disc overlay (CO) (2-cm diameter, 1.5-mm thick; IPS e.max, A2 shade, Ivoclar Vivadent, Schaan, Liechtenstein) was selected to model an indirect restorative material through which irradiating light would pass.

Base and catalyst pastes of each product were equally dispensed on a glass plate and were hand-mixed using a metal spatula. The mixture was then applied to the horizontal diamond element of an attenuated total reflectance (ATR) unit attachment (Golden Gate, Specac, Woodstock, GA, USA) in the optical bench of a Fourier transform infrared spectrometer (Tensor Series, Bruker Optik GmbH, Ettlingen, Germany). For groups simulating tooth temperature, the diamond surface temperature was elevated to 28°C or 32°C using a custom-made heating device, and the surface temperature was constantly monitored using a K-type thermocouple (SmartMether, Novus, Porto Alegre, Brazil) during resin cement polymerization. Before resin placement, adhesive tape (3M ESPE) was placed around the diamond surface to act as a spacer, ensuring standard thickness for all specimens (100-120 μm).

The deposited cement was covered with a Mylar strip and polymerized using one of three curing modes. Specimens were exposed directly to light activation without any overlying restorative material (DLE or control group) for 20 seconds for MX and 40 seconds for U100 (manufacturers' instructions) to a blue light-emitting diode light-curing source emitting 1500 mW/cm^2 (Radii Plus, SDI Limited, Victoria, Australia). Seven replications were made for each test condition (n=7) based on previous studies using the same methodology.[6,21-23] To ensure delivery of consistent levels of irradiance during testing, the output of the light was continuously checked during testing, using a handheld dental curing radiometer (Cure Rite, Dentsply Caulk, USA). The distal end of the light-emitting guide was placed directly against a 1-mm-thick glass slide, positioned directly over top of the Mylar-covered resin specimen. When exposing cements to light by overlying a ceramic disc, the disc was placed directly between the Mylar sheet and the light-emitting guide end (Figure 1). In addition, specimens were allowed to self-cure without light exposure.

Degree of Conversion

Infrared (IR) spectra were collected between 1680 and 1500 cm^{-1} at a rate of one spectrum per second (16 scans per spectrum) at 4 cm^{-1} resolution. Data were counted from the moment the IR scan demonstrated that the resin was stabilized on the ATR

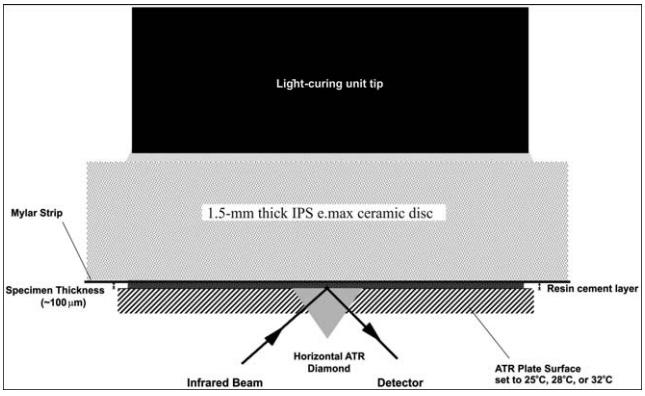

Figure 1. *Illustrative diagram demonstrating the interaction between the infrared beam and the specimen, as well as the position of the 1.5-mm thick ceramic disc and the light-curing unit tip.*

Table 2: *Mean (SD) Direct Conversion Values of MX at Room (25°C) and Tooth Temperatures (28°C and 32°C)*[a]

Temperature at Polymerization	Direct Light Exposure	Indirect Ceramic Disc Overlay	Self-curing
25°C	58.1 (2.7) Ba	52.4 (1.3) Cb	47.6 (1.8) Bc
28°C	66.2 (2.7) Aa	59.5 (2.4) Bb	61.8 (3.2) Aab
32°C	70.9 (2.0) Aa	66.2 (3.3) Aab	62.1 (1.4) Ab

[a] Mean *values with similar letters (upper case within column; lower case within row) are not significantly different (*$p>0.05$*).*

surface and any overlying objects had been placed. Spectra were recorded continuously during each 1-second interval for 12 minutes to generate kinetic spectra of monomer conversion. Monomer conversion was calculated using standard methods that evaluated changes in the ratios of aliphatic-to-aromatic C=C IR absorption peaks (1636 cm^{-1}/1608 cm^{-1}) in the uncured and cured states,[24,25] according to the following equation:

$$DC = 1 - \frac{(C=C_{aliphatic}/C=C_{aromatic})\,polymer * 100}{(C=C_{aliphatic}/C=C_{aromatic})\,monomer}$$

Where DC is the degree of conversion, $C=C_{aliphatic}$ corresponds to the IR absorbance of aliphatic double carbon bonds, and $C=C_{aromatic}$ is the IR absorbance of aromatic carbon bonds.

Before determining conversion, calibration graphs were made relating the absorbance ratios of known molar concentrations of aliphatic and aromatic C=C to their respective absorbance height ratios as previously established.[25] Conversion values among all curing modes were compared statistically within each product at 12 minutes from the time the resin cement was stabilized on the ATR surface. All polymerized specimens were carefully removed from the ATR plate and measured for thickness to the nearest 0.01 mm using a digital micrometer (Series 406; Mitutoyo America Corp, Aurora, IL, USA) to ensure similar thickness among all specimens.

Statistical Analyses

DC data were evaluated within each dual-cure resin product using a two-way analysis of variance (ANOVA) (factors: temperature [three levels] and curing condition [three levels]) followed by Tukey's post-hoc test. Direct comparison of conversion values between products was not made, because such comparisons can only be validly made when resins have the same chemical formulation. All testing was performed at a preset $\alpha=0.05$ using personal statistical software (SAS 8.0 for Windows; SAS Institute Inc, Cary, NC, USA). Post-hoc power analysis was performed for the statistical analysis of degree of conversion data using additional software (Statistics 19, SPSS Inc, IBM Company, Armonk, NY, USA).

RESULTS

Degree of Conversion

The study was adequately powered for both factors, temperature and curing mode (over 95%; $\alpha=0.05$). The two-way ANOVAs indicated that the interaction between curing mode and temperature was a significant factor in affecting DC for both MX ($p=0.0098$) and U100 ($p<0.00001$).

Tables 2 and 3 present DC of dual-cure SARCs exposed using the three curing conditions at three temperatures. Temperature increases from 25°C to 28°C resulted in a significantly higher DC in all groups of MX (Table 2), while a significant increase in DC of U100 (Table 3) was only observed in groups exposed to the CO condition and in the self-curing groups ($p<0.0001$). No significant increase in DC was observed in the DLE group when the temperature increased from 25°C to 28°C. U100 exposed at 32°C showed higher DC values than when it was exposed to lower temperatures ($p<0.00001$).

For MX, the effect of different curing conditions on DC was temperature-dependent. When exposed to tooth temperatures, the self-curing mode provided similar DC values to those when curing was through the CO. The self-polymerizing mode at 25°C led to

Table 3: *Mean (SD) Direct Conversion Values of U100 Polymerized at Room (25°C) and Tooth Temperatures (28°C and 32°C)*[a]

Temperature During Polymerization	Direct Light Exposure	Indirect Ceramic Disc Overlay	Self-curing
25°C	49.9 (1.0) Ba	45.1 (2.6) Cb	25.2 (3.7) Cc
28°C	50.7 (2.3) Ba	46.8 (1.0) Bb	29.5 (1.8) Bc
32°C	57.4 (0.9) Aa	47.9 (1.5) Ab	34.1 (1.6) Ac

[a] *Mean values with similar letters (upper case within column; lower case within row) are not significantly different (*$p>0.05$*).*

lower DC values than did the attenuated light-activated mode ($p=0.0427$). On the other hand, the effects of curing conditions on DC values were not influenced by temperature when U100 was evaluated. The DLE groups demonstrated higher DC values than groups exposed to light attenuated by the ceramic overlay ($p<0.0001$). The self-cure mode led to the lowest DC values in all temperatures for this material.

Figures 2 and 3 show the polymerization kinetic of representative specimens, representing the pattern observed in most specimens regarding the effects of tooth temperature on the time-based conversion profiles of U100 and MX, respectively. All Figures included the comparison of these values to the 12-minute degree of conversion value from the DLE group at 25°C (dashed line) as a control. At 28°C and 32°C (Figure 2), time-based conversion changes in the CO condition of U100 exhibited polymerization kinetics similar to that obtained in the room-temperature DLE groups at 12 minutes. The polymerization kinetics of the CO groups exposed to tooth temperatures showed higher slopes during the initial stages of polymerization, indicating higher rates of conversion, until the light curing unit shut off, in comparison to the polymerization kinetics observed at room temperature, where the slopes of these time-based conversion values were much lower. Then, a slow, continual increase was noted in all CO groups. The slowest initial curing rate was noted for the self-cure only mode at room temperature, resulting in the lowest DC values. The effects of temperature were more evident in the self-cure groups, as the conversion rate at tooth temperatures was apparently much faster than that at room temperature. Less time was required for the self-curing mode of Rely X U100 to begin polymerization when it was exposed to tooth temperatures.

Overall, the time-based conversion profile of MX appeared to be more influenced by tooth temperatures than U100 was. Interestingly, the basic profile of time-based conversion for the CO groups was similar to that of the self-curing only group, indicating that the overall polymerization reaction, when curing through the ceramic disc, was related to the self-cure reaction. At room temperature, the CO group exhibited higher slope at the beginning of polymerization in comparison to the CO group at 28°C, indicating higher initial curing rates at room temperature. The time-based conversion profile of the self-curing mode of MX apparently provided faster curing rates than did U100, regardless of temperature. The polymerization of MX at simulated tooth temperatures promoted faster curing rates than polymerization occurring at room temperature in groups relying solely on self-curing, which led to 12-minute DC values as high as those at room temperature.

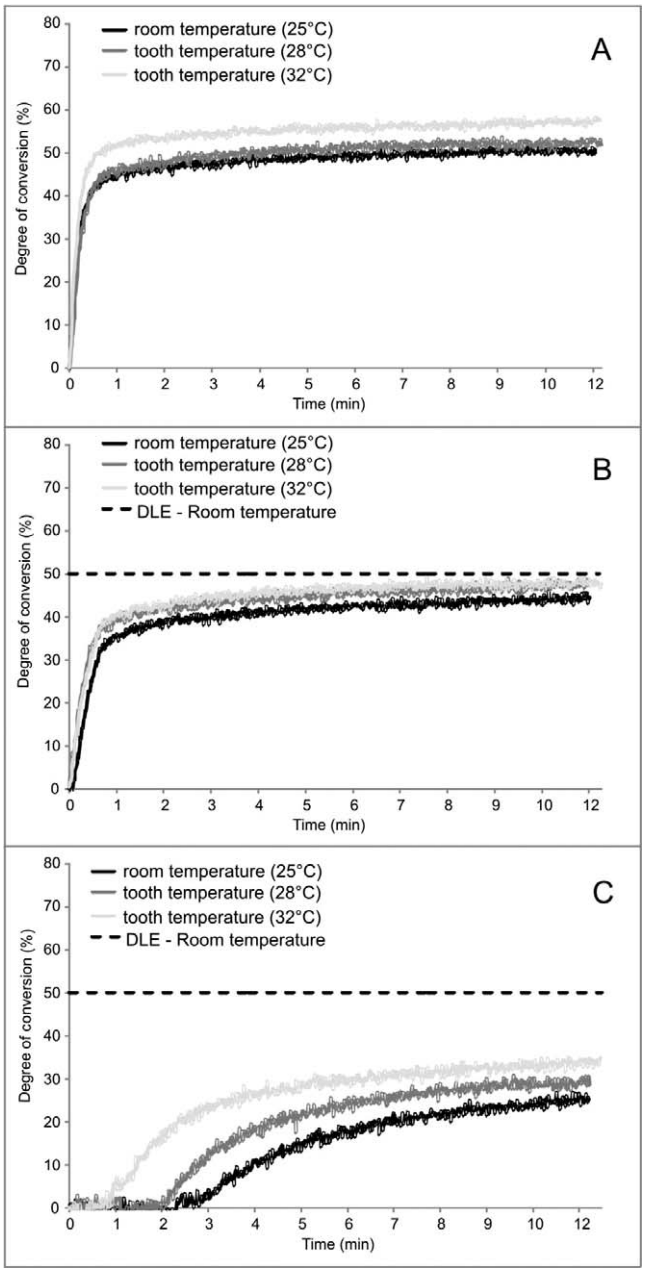

Figure 2. *Polymerization kinetics of U100 at 25°, 28°, and 32°C exposed to direct light exposure (DLE) (A), light exposure through ceramic disc overlay (CO) (B), and self-curing (SC) (C). The dashed line represents the degree of conversion after direct light exposure at 25°C.*

DISCUSSION

The results validated the first research hypothesis, which anticipated that tooth temperature increases

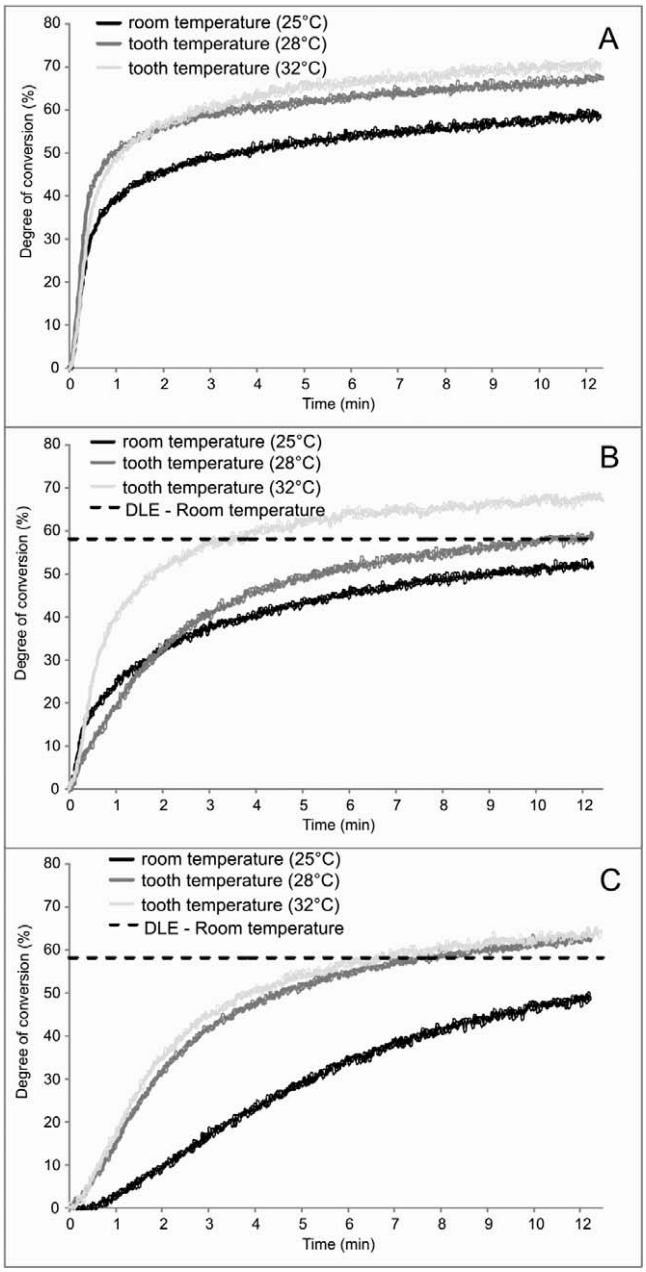

Figure 3. *Polymerization kinetics of MX at 25°, 28°, and 32°C exposed to 3 curing conditions: direct light exposure (DLE) (A), light exposure through ceramic disc overlay (CO) (B), and self-curing (SC) (C). The dashed line represents the degree of conversion after direct light exposure at 25°C.*

DC of SARCs using simulated clinical conditions. Because the products tested are relatively new and involve acid-base reactions and radical polymerization, it is difficult to anticipate the effects of such increases in DC on cement mechanical properties and bonding to dentin. It has been shown that high temperature can catalyze the acid-base reaction between acid groups and calcium or aluminum from fillers to increase pH.[19] As a consequence, the pH would rise more quickly at a high temperature than at room temperature. Therefore, polymerization would proceed without the well-known detrimental effect from low pH[21,26] but could, for this reason, compromise bonding between such products and dentin. However, it should be pointed out that studies evaluating the effects of temperature on SARCs have focused on a temperature of 60°C,[19] which is considerably higher than that of the tooth. Thus, it is expected that tooth temperatures may increase the initial pH of these products more quickly than when these products set at room temperature. On the other hand, it is reasonable to expect that such effects caused by the cement exposure to tooth temperature are less pronounced than the effects on pH increase observed when such products are exposed to high temperature.

The self-curing mode of SARCs at room temperature was less effective in elevating DC than were the light-activating modes, corroborating previous findings.[6,10,27] This curing mode was also more influenced by temperature ranges than light-activated modes. More specifically, the DC of MX in self-curing mode increased approximately 14.2% from 25°C to 28°C, while the light-activated groups showed an approximately 7.1% to 8.1% increase within the same temperature range. For U100, self-curing mode groups increased 4.3% from 25°C to 28°C, while light-activated modes increase approximately 1.8%. This finding demonstrates that the effectiveness of self-curing components in SARCs exposed to tooth temperature is not as poor as that observed at room temperature. However, it should be noted that Rely X U100 still led to low DC values, even at 32°C. In addition, these results help explain those observed by Schmid-Schwap and others,[28] who evaluated the cytotoxicity of conventional and self-adhesive resin cements. In that study, U100 was one of the most cytotoxic cements when its effect was evaluated 10 minutes after it was allowed to self-cure.

The current results demonstrated that, regardless of the temperature, both curing conditions that simulated the cementation of an overlay or the complete absence of curing light led to lower DC values than those obtained from the control group, in which the SARCs were directly exposed to curing light. Such differences were observed when all experimental groups of U100 were compared with the DLE groups. For MX, only two curing conditions led to no significant differences in DC values, in comparison to the values from DLE groups: light activation through ceramic overlay at 32°C and self-

cure at 28°C. Thus, the data invalidated the second research hypothesis for MX and U100, which anticipated that DC values will not be significantly different from those obtained when exposing the specimen through a porcelain overlay or when allowed to totally self-cure without light. It should be mentioned that most statistical comparisons were made within each temperature. A similar increase in DC values was also observed in DLE groups, the differences between control group and experimental groups within each temperature remained constant. Despite such differences, when SARCs were exposed to attenuated light (CO groups) or allowed to self-cure at simulated tooth temperature, their DC values were apparently as high as those of the control (DLE) group at room temperature.

Although the comparisons between time-based conversion profiles (Figures 2 and 3) were not based on statistical methods but only on visual analyses, the effects of tooth temperature on polymerization kinetics were apparently more pronounced when the SARCs were allowed to self-cure. Besides, and still based on the conversion profiles, the increase in temperature also reduced the delay in the polymerization start. Based on this evidence, two assumptions can be made: (1) despite the lack of information from the manufacturers, MX presents higher amounts of self-curing components than U100; and (2) the differences in DC and conversion profiles caused by the increased temperature were apparently related to the amount of self-curing component in each resin cement. The degradation rate of self-curing components into radicals increases with increased temperature,[29] so radicals are created more rapidly when heated. For MX at 25°C (Figure 3A), the importance of self-curing components to compensate for lower light levels reaching the resin cement was evident, as a significant increase in conversion values with time was noted after light exposure. On the other hand, the light-activating components in U100 that are responsible for compensating for light attenuation were seen to work more effectively than in MX. For instance, at 25°C, the conversion-based profile using a ceramic disc placed over U100 produced nearly the same result as when the SARC was exposed to unattenuated light.

Time-based conversion profiles also demonstrated that the increase in DC values in all curing conditions is a consequence of increased polymerization rate (Figures 2 and 3). In this type of resin cement, a high polymerization rate can have detrimental effects on bonding to dentin, because higher shrinkage stress is developed as a consequence.[18] Also, high polymerization rates enhance cross-link density and network quality,[30] resulting in a reduction of resin matrix permeability for fluoride ion release from the SARC.[31,32] As a consequence, SARCs polymerized at tooth temperature may release fewer fluoride ions than they do at room temperature, so the clinical benefits of fluoride release may be compromised as well. Conversely, the consequent high DC may improve mechanical properties.[3,30] Based on such assumptions, it is reasonable to assume that bonding and longevity of indirect restorations cemented with SARCs may be influenced by tooth temperature. However, only further studies evaluating short- and long-term mechanical properties and bond strength of such products to dentin and enamel might confirm how positive or negative such an influence would be.

Curiously, when light-activation of MX was performed through a CO, the conversion profiles at room temperature showed an apparently higher slope in the curve at the initial conversion than did specimens heated to 28°C. This finding indicates that the resin cement exhibited a higher curing rate at room temperature than at 28°C in this curing condition. It is important to point out that the moment of initial polymerization corresponds to the moment when the resin cement is being exposed to light from the curing unit. Therefore, based on the monomer conversion versus time plot, it seems that light activation was more effective at room temperature than at 28°C. Because the same energy density was delivered to the resin cement in both conditions, such a difference in the time-based conversion profile at the beginning of the polymerization may be related to the effects of tooth temperature on the self-curing components. As discussed earlier, because the degradation rate of such components into radicals increases greatly with increased temperature, the radicals are created more rapidly when heated. Conversely, it seems that such a premature activation of self-curing components might have interfered with components used in the light activation process.

The resin cement polymerization was evaluated for 12 minutes, which corresponds approximately to the time spent by clinicians to cement an indirect restoration to a prepared tooth. Therefore, the exposure of SARCs to tooth temperatures will allow the cement to be better polymerized at the moment of occlusal adjustments and first occlusal stress. However, it must be emphasized that further polymerization, along with an additional acid–base setting reaction, may proceed during the following

24 hours.[2,33] For this reason, no assumptions can be made regarding long-term bonding and resin cement mechanical properties, so only evaluation of SARCs after all curing reactions are completed can provide evidence of how tooth temperature affects their final mechanical properties and tooth-bonding capabilities.

CONCLUSIONS

Within the limitations imposed by the current study, the following conclusions may be made:

1. SARCs allowed to polymerize at temperatures simulating that of a prepared tooth (28°C or 32°C) showed increased monomer conversion values compared with the same products polymerized at room temperature (25°C).
2. At temperatures similar to those of the prepared tooth surface, SARCs exposed to attenuated light through the thickness of an indirect ceramic restoration exhibited degree of conversion values as high as those observed when resin cements were exposed to direct light at room temperature.
3. Despite the significant increase in DC promoted by polymerization at simulated prepared tooth temperatures for self-cured SARCs, the effectiveness of self-curing components at higher temperatures, in comparison to dual-curing polymerization at room temperature, was product-dependent.

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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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Influence of Application Techniques on Contact Formation and Voids in Anterior Resin Composite Restorations

SR Kwon • U Oyoyo • Y Li

Clinical Relevance

Diastema closure with direct resin composites should be free of voids and establish proper contact formation. Good results can be achieved with the pull-through technique and the use of prefabricated matrices.

SUMMARY

This study evaluated the influence of three different application techniques on contact formation and voids in anterior resin composite restorations. Artificial ivorine teeth were randomly assigned to three experimental groups, with 20 specimens in each group. One operator performed all restorations using the Teflon tape, pull-through, or bioclear matrix technique. The treatment time required for each restoration was recorded. An examiner blinded to the treatment group performed the visual evaluation of six criteria, including proper contact formation. The restored teeth were cut to yield a total of 180 sections for microscopic evaluation. The Kruskal-Wallis procedure was performed to evaluate the significance of treatment time, number of voids, percent porosity area, and void diameter. There were significant differences in treatment time among the three groups ($p<0.05$). The bioclear matrix technique required the least time for the treatment of one diastema closure ($p<0.05$). The Teflon tape technique resulted in proper contact formation in 80% of specimens, a rate that was significantly lower than that associated with the pull-through and bioclear matrix techniques ($p<0.05$). Out of 540 imaging areas 160 images were free of voids. The number of voids and the percent porosity area were not significantly different among the three techniques ($p>0.05$). However, the mean void diameter was greater with the bioclear matrix technique compared to the other two techniques ($p<0.05$).

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INTRODUCTION

Diastema closure with direct resin composite restorations can be a conservative treatment modality to improve the appearance of a smile. Direct resin composite bonding may lack the permanence of indirect veneers and full coverage restorations. However, conservation of sound tooth structure, shorter treatment time, ease of repair, and the low cost of the treatment compared to other treatment modalities are distinct advantages of direct bonding.[1]

Diastema closures in the anterior dentition can be accomplished without the use of a matrix or wedge, thus providing better control of proximal contours in the gingival embrasure areas.[2] In these cases, a Teflon tape is commonly used to prevent bonding to the adjacent tooth. A tight and properly contoured contact can also be achieved using a "pull-through" matrix technique, whereby a strip is pulled from the facial toward the lingual to push the resin material through the proximal to form a smooth contour adapted properly to the adjacent tooth.[1,3] Another approach is to use custom fabricated matrices with a combination of flowable composite and a syringeable composite filling technique.[4]

Success with resin composite restorations requires a disciplined technique, and the restorative dentist seems to have the greatest effect on longevity.[5] Proper contact formation and margin adaptation may be considered as critical steps in the treatment of space closure using direct resin composite restorations. The use of a proper application technique should ideally establish a tight contact and a restoration free of voids and porosities.

The influence of various application procedures on cavity wall adaptation and void formation has been investigated on posterior Class I and Class II cavities.[6-9] This is an important issue since many studies consider the presence of voids and porosities to be disadvantageous to the restoration. First, voids compound the problem of producing a smooth surface to a composite restoration.[10] Second, voids result in a lower resistance to fatigue that may influence durability.[11] Third, flaws introduced during the clinical handling of resin composites will have a negative effect on the flexural strength of the material.[12] Fourth, increased void content leads to an increase in polymerization shrinkage, predisposing restorations to marginal leakage, staining, and secondary caries.[13]

The injection of a syringeable composite has been reported[7,8] to result in a better restoration with less voids compared to a packing technique with a highly viscous composite in posterior Class I restorations. The use of uncured flowable composite with a layer of medium-viscous composite injected—referred to as the "snow-plough technique"—produced the most homogeneous restoration in small posterior cavities.[9] However, there is a lack of information on the comparison of different application techniques with regard to the formation of voids in anterior restorations. Thus, the purpose of this study was to evaluate the influence of three different application techniques on the contact formation and voids in anterior resin composite restorations on simulated diastemas in artificial ivorine teeth. The null hypotheses to be tested were that there would be no differences among the three techniques in 1) the treatment time required, 2) establishment of proper contact and contour, and 3) void formation within the resin composite restorations.

MATERIALS AND METHODS

Specimen Preparation

Sixty artificial ivorine teeth (#9) were marked with a pencil to enable standardization of a 1.5-mm mesial diastema when trimmed with a model trimmer (Orthodontic model trimmer, Whip MixAE, Louisville, KY, USA). In order to facilitate bonding, air-polishing (Prophy-Jet, Dentsply, York, PA, USA) was performed on the trimmed surface. The teeth were randomly assigned to three experimental groups, with 20 specimens in each group, and mounted on a phantom-head dentoform (Columbia Dentoform Corp, Long Island, NY, USA).

Application Techniques

Table 1 summarizes the step-by-step procedure for the three different application techniques, and Table 2 summarizes the restorative materials used.

Group A (Teflon Tape Technique)—The adjacent tooth (#8) was wrapped around with a Teflon tape (Teflon plumbers tape, Westbrass, Los Angeles, CA, USA) to prevent inadvertent bonding. A one-component, self-etching, light-cured dental adhesive (Bond Force Kit, Tokuyama Corporation, Tokyo, Japan) was applied and rubbed onto the surface (20 seconds) and light-cured (20 seconds). A syringeable composite (Estelite Omega PLT, Tokuyama Corporation) was injected from the labial and the lingual and sculpted with a composite placement instrument (Gold Microfil, Almore International Inc, Portland, OR, USA), followed by light-curing for 40 seconds with an LED light-curing unit (Valo-light: 1717 mW/

Table 1: *Step-by-Step Protocol by Application Technique*

Group	Step-by-Step Protocol
A: Teflon tape technique	1. Teflon tape is placed around #8
	2. Resin adhesive is applied on #9 and light-cured (LC)
	3. Resin composite is placed on #9, sculpted, and LC
	4. Teflon tape is removed from #8
	5. Gingival margin is shaped with #12 scalpel blade
	6. Restoration is finished and polished
B: Pull-through technique	1. Resin adhesive is applied on #9 and LC
	2. Celluloid strip is placed and resin composite applied on #9 and sculpted
	3. Strip is pulled from lingual to facial, composite contoured with brush, and LC
	4. Celluloid strip is placed and resin composite applied and sculpted
	5. Strip is pulled from facial to lingual, composite contoured with brush, and LC
	6. Restoration is finished and polished
C: Bioclear matrix technique	1. Bioclear diastema closure matrix is placed mesially to #9
	2. Resin adhesive is applied on #9 but not LC
	3. Flowable composite is injected into the cervical area and then LC
	4. Tooth separation is created with a wedge
	5. Adhesive resin, flowable composite, and paste composite are placed and LC
	6. Bioclear diastema closure matrix is removed
	7. Restoration is finished and polished

cm^2; Ultradent Products Inc, South Jordan, UT, USA). The restoration was finished with contouring discs (Soflex kit, 3M, St Paul, MN, USA) and strips (Epitex strips, GC America Inc, Alsip, IL, USA).

Group B (Pull-Through Technique)—On application of the syringeable composite, the bulk of composite was first pulled from the lingual to the facial with a celluloid strip (Celluloid strip, GC America Inc). After sculpting and light-curing the procedure was repeated from the facial toward the lingual. The restoration was sculpted, light-cured, and finished.

Group C (Bioclear Matrix Technique)—A prefabricated matrix (Bioclear diastema closure matrix, Bioclear, Tacoma, WA, USA) was stabilized into the gingival area. A flowable composite (Estelite Flow Quick, Tokuyama Corporation) was injected into the cervical area and light-cured. Tooth separation was performed with a wedge (G-Wedge, Garrison Dental Solutions, Spring Lake, MI, USA). A small amount of flowable resin was injected followed by a syringeable composite to fill the matrix and the restoration was light-cured. The matrix was gently removed and the restoration finished.

One operator with 20 years of clinical experience performed all 60 restorations. The application and finishing time required for each restoration were recorded.

Visual Evaluation of Contact Areas, Embrasures, and Line Angles

The evaluation criteria for proper formation of contact areas, embrasures, and line angles are summarized in Table 3. An examiner blinded to the treatment group performed the evaluation of the six criteria with a dental loupe (2.5× magnification). Each criterion was determined to be either correct or an error, with one point given to a correct criterion.

Microscopic Evaluation of Porosity Formation

On completion of the visual evaluation tooth #9 was removed from the model and mounted on a sectioning machine (TechCut 4, Allied High Tech Products Inc, Compton, CA, USA) to prepare three 0.5-mm sections representative of the incisal, middle, and gingival areas. Figure 1 illustrates the location of the sections relative to the incisal edge and the labial cervical line. The sections were sequentially polished with special silicon carbide grinding papers (Microcut, grit 400, 600, and 1200; Buehler, Lake Bluff, IL, USA) to a thickness of approximately 200 μm.

A total of 180 polished sections were viewed under the microscope at 20× magnification (Olympus BH2, Olympus Corporation, Tokyo, Japan), and images were taken at three different areas per section. Figure 2 illustrates the imaging location. Each area was measured for number of voids, void diameter, total viewing area, and total porosity area with image J analysis software (National Institutes of

Table 2: *Restorative Materials Used in this Study*

Material	Manufacturer	Mean Filler Size, nm	Filler Content, vol%	Color	Lot No.
Bond Force Kit	Tokuyama Corporation, Tokyo, Japan	—	—	—	108EY2
Estelite Flow Quick	Tokuyama Corporation, Tokyo, Japan	300	53	A1	070EY2
Estelite Omega PLT	Tokuyama Corporation, Tokyo, Japan	200	71	EA1	006E41

Table 3: *Evaluation Criteria for Contact Area, Embrasures, and Line Angles*

Criteria	Correct	Error
Mesial contact	Visual contact, correct width and position, tight	Wide narrow, too incisal, too gingival, too facial, too lingual, irregular, loose
MF embrasure	Normal contour following adjacent tooth	Closed, open, irregular
MF line angle	Correct shape and position	Too straight, too angled, too sharp, too round
MG embrasure	Normal contour following adjacent tooth	Closed, open, irregular
MI embrasure	Normal contour following adjacent tooth	Closed, open, irregular
ML embrasure	Normal contour following adjacent tooth	Closed, open, irregular

MF: mesio-facial; MG: mesio-gingival; MI: mesio-incisal; ML: mesio-lingual

Health, Bethesda, MD, USA) (Figure 3). The percent area of porosity was calculated with the following equation:

$$\text{Percentage (\%) porosity} = \text{total porosity area} / \text{total viewing area} \times 100.$$

As a control procedure five preloaded composite tips and one flowable composite syringe were evaluated for porosities. Five millimeters of the tip was removed with a blade and the composite inside was light-cured for 60 seconds. The cured composite was removed from the tip or the syringe, sectioned, and polished to prepare three 200-μm–thick sections for microscopic evaluation.

Statistical Analysis

The nonparametric Kruskal-Wallis procedure with Bonferroni pairwise post hoc comparisons was performed to evaluate the distribution of the total treatment time, number of voids, percent porosity area, and mean void diameter. Chi-square tests were used to compare the proportion of samples demonstrating contact among the three techniques. All tests of significance were two-sided and were conducted at an alpha level of 0.05 with SAS v 9.1.3 (SAS Institute, Cary, NC, USA).

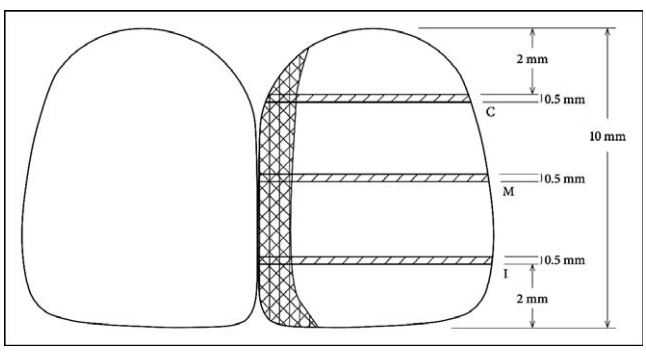

Figure 1. *Design of diastema closure and sectioning of tooth.*

RESULTS

Treatment Time

Kruskal-Wallis test followed by pairwise comparisons showed significant differences in application, finishing, and total treatment times among the three groups ($p<0.05$) (Table 4). The bioclear matrix technique required the least time for the treatment of one diastema closure ($p<0.05$).

Visual Evaluation of Contact Formation

The Teflon tape technique demonstrated contact formation in 80% of specimens, which was significantly lower than in 100% of the pull-through and bioclear matrix technique specimens ($p<0.05$) (Table 5). The embrasure and line angle criteria produced almost no errors with all three techniques so no statistical analysis was performed.

Evaluation of Porosity Formation by Technique

Microscopic evaluation of the composite sections taken from five preloaded tips and one flowable composite syringe showed that the materials were free of porosities. The voids in the restorations were spherical in shape and occurred mainly along the

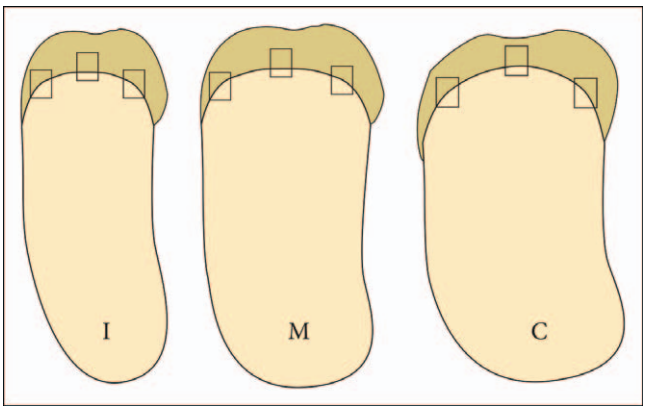

Figure 2. *Imaging areas on sections.*

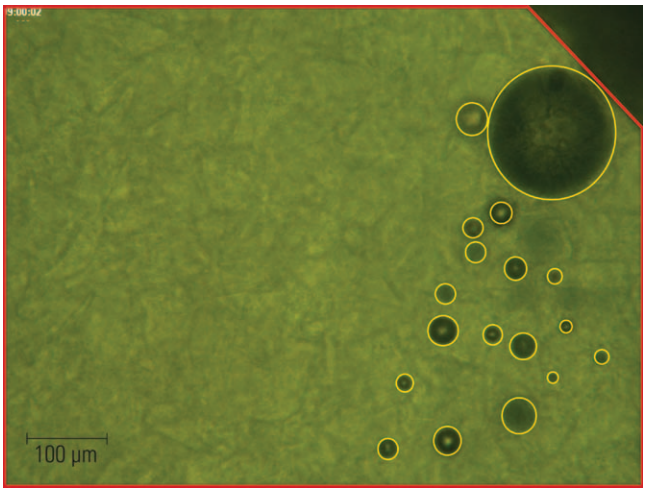

Figure 3. *Calculation of total viewing area (red) and total porosity area (yellow) with Image J software.*

artificial tooth and adhesive/resin composite interface (Figure 4).

Out of 540 imaging areas 160 images were free of voids. The number of voids and the percent porosity area were not significantly different among the three techniques ($p>0.05$). However, the mean void diameter was greater with the bioclear matrix technique compared to the Teflon tape and pull-through techniques ($p<0.05$). The mean values and standard deviations (SDs) of porosity data by technique and location are summarized in Table 6 and illustrated as box plots in Figures 5 through 7.

DISCUSSION

This phantom-head dentoform setup in a clinical laboratory reflects the porosities related to different techniques under ideal circumstances and indicates that the clinical handling with challenges of the oral environment may result in different, less favorable results.

The composites selected for this study were shown to be free of porosities when cured from within the syringe or tip. This finding is in accordance with

Table 4: *Application, Finishing, and Total Treatment Time Values [Mean (standard deviation [SD])] in Minutes, by Technique*[a]

	A: Teflon Tape	B: Pull-Through	C: Bioclear Matrix
Application time	8.9 (1.7) A	12.5 (1.7) B	6.1 (1.2) C
Finishing time	4.8 (0.6) A	2.3 (0.4) B	5.3 (0.7) A
Total treatment time	13.6 (1.8) A	14.7 (1.7) A	11.4 (1.3) B

[a] *Within a row, same letters indicate means that are not statistically different (p>0.05).*

Table 5: *Frequency of Contact Formation by Technique*[a]

	Technique			Total
	A: Teflon Tape	B: Pull-Through	C: Bioclear Matrix	
No contact	4	0	0	4
Contact	16	20	20	56
Total	20	20	20	60

[a] *Pearson chi-square = 8.571 (df=2),* p = *0.014.*

those of the study of Opdam and others,[8] in which the unused tip of a composite was used as the control. However, other studies[10,14,15] in which the composite material was extruded from the syringe showed microporosities. The authors of these studies stated that voids are mainly introduced as the material is extruded through the orifice of the syringe.

This study measured the number of voids, percent porosity area, and the void diameter. The mean number of voids and the mean percent porosity area ($\asymp$1.0%) were relatively small so that homogeneous restorations could be achieved with all three techniques used.

Clinically, the void diameter can be considered most important, since large voids may lead to a lower resistance to fatigue and decreased wear resistance.[8] Interestingly, there was no difference among the three techniques in terms of the number of voids and the percent porosity area, whereas the bioclear matrix technique exhibited significantly larger voids in diameter. One explanation for this difference may be the use of a combination of flowable and syringeable composite in the middle area that may be more prone to air entrapment, resulting in the

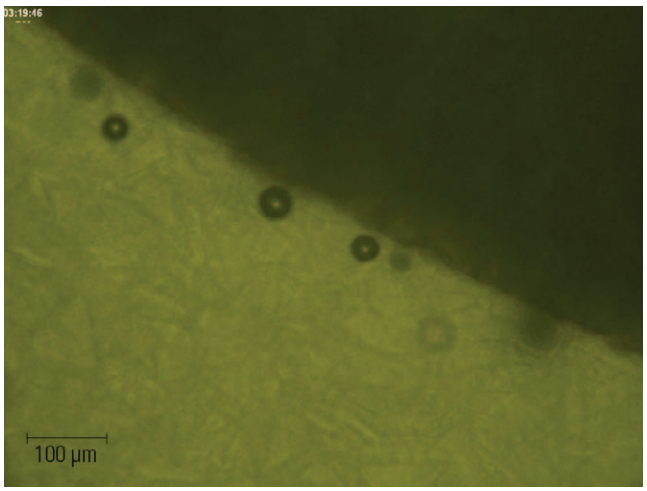

Figure 4. *Voids along tooth and adhesive/restoration interface.*

Table 6: *Number of Voids, Percent Porosity Area, and Void Diameter [Mean (standard deviation [SD])] by Technique and Location*[a]

	Location	A: Teflon Tape	B: Pull-Through	C: Bioclear Matrix
Number of voids	Incisal	2.22 (3.64)	2.40 (2.90) A	2.65 (2.58) A
	Middle	2.62 (3.04) a	4.27 (4.82) bB	4.22 (3.39) bB
	Cervical	2.67 (2.61)	2.58 (2.08) A	1.88 (2.57) A
	Total	2.50 (3.12)	3.08 (3.55)	2.92 (3.02)
Percent porosity area	Incisal	0.94 (1.54)	0.86 (1.15)	1.10 (1.27) A
	Middle	1.02 (1.16) ab	0.92 (1.11) a	1.49 (1.38) bA
	Cervical	0.95 (1.11)	1.14 (2.00)	0.70 (1.12) B
	Total	0.97 (1.28)	0.97 (1.47)	1.10 (1.29)
Void diameter, μm	Incisal	26.58 (33.46) a	26.96 (28.20) a	37.91 (33.46) bA
	Middle	31.95 (25.07) a	26.63 (23.19) a	43.40 (31.20) bA
	Cervical	30.10 (27.85)	31.73 (27.22)	25.56 (32.06) B
	Total	29.54 (28.93) a	28.43 (26.25) a	36.62 (32.93) b

[a] *Within rows, different lowercase letters indicate means that are statistically different ($p<0.05$); within columns, different uppercase letters indicate means that are statistically different by location ($p<0.05$).*

formation of larger voids with the bioclear matrix technique. This explanation is contrary to the results in occlusal preparations, in which the use of the "snow-plough technique" produced the most homogeneous restoration.[9] This discrepancy might be explained by the ability to apply pressure and by the better access to Class I cavities, whereas there is only limited access of the syringe in the anterior region, limited partly by the prefabricated matrix.

The use of a small amount of composite rolled into the shape of a ball and subsequently applied with a composite applicator may facilitate access and reduce the void diameter when using the bioclear matrix technique.

Opdam and others[7] showed differences in porosity within the composite by the type of operator. The dental student operator produced the best results. The authors speculated that this was due to the fact that students are more likely than general practitioners to follow the protocol. In our study, one operator performed all of the restorations, which also explains the relatively small SDs in treatment time and percent porosity area. Further studies should be performed to evaluate which technique is more

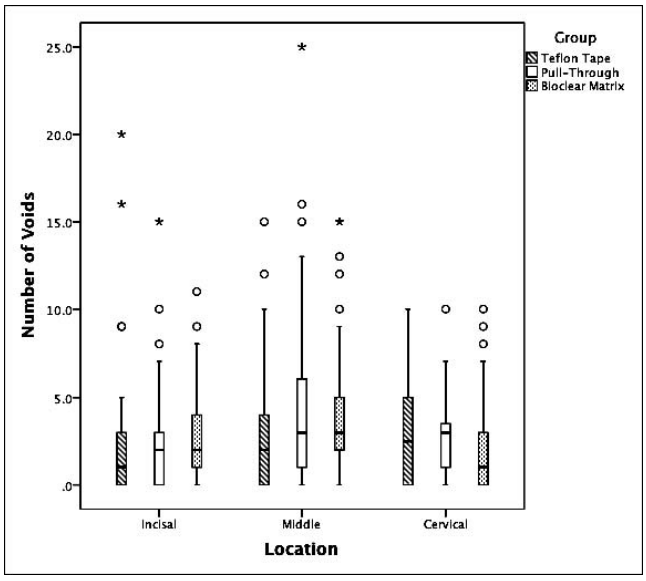

Figure 5. *Box plots of number of voids by technique and location (o, outliers; *, extreme data points).*

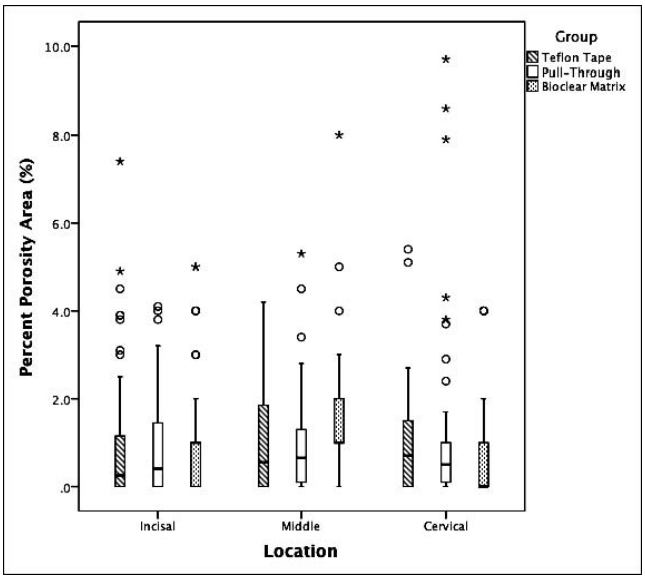

Figure 6. *Box plots of percent porosity area by technique and location (o, outliers; *, extreme data points).*

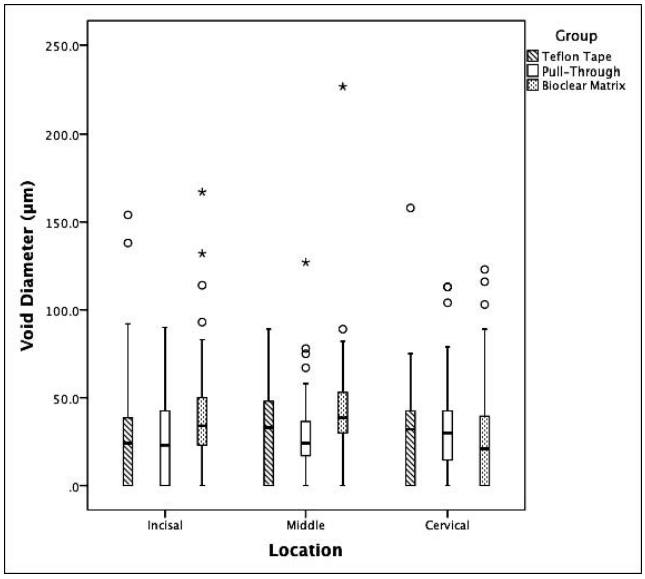

Figure 7. *Box plots of void diameter by technique and location (o, outliers; *, extreme data points).*

easily learned and applied by operator type. Future studies may also include different types of application techniques that have not been addressed in this study.

Proper contact formation is essential for the esthetics and function of a diastema closure. The frequency of contact formation was less with the Teflon tape technique compared to the other techniques. We suggest pre-wedging to improve the outcome with the Teflon tape technique. The dentoform setup is limited in simulating the elasticity of periodontal ligaments *in vivo*. However, the artificial gingiva and minute movement of artificial teeth in the socket enable the use of pre-wedging.

Based on the results, the treatment time for a single diastema closure was significantly smaller using a prefabricated matrix, leading to the rejection of our first null hypothesis. The second null hypothesis was rejected, since the pull-through and bioclear matrix techniques provided better contact than did the Teflon tape technique. The third null hypothesis was partly rejected, as the void diameter in the composite restoration was larger in the bioclear matrix group compared to the other two technique groups.

CONCLUSIONS

Diastema closure with direct resin composites should be void-free and should establish proper contact formation. The results of this study showed that void-free restorations were difficult to obtain. However, all application techniques used produced relatively homogeneous restorations. The best results in contact formation could be achieved with the pull-through and bioclear matrix techniques.

Acknowledgements

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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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Evaluation of Dental Restorations: A Comparative Study Between Clinical and Digital Photographic Assessments

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Clinical Relevance

The digital photographic method is a useful tool for assessing the quality of dental restorations, providing information that goes unnoticed with the visual-tactile clinical examination method.

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SUMMARY

The aim of this study was to compare the efficacy of a direct clinical evaluation method with an indirect digital photographic method in assessing the quality of dental restorations. Seven parameters (color, occlusal marginal adaptation, anatomy form, roughness, occlusal marginal stain, luster, and secondary caries) were assessed in 89 Class I and Class II restorations from 36 adults using the modified US Public Health Service/Ryge criteria. Standardized photographs of the same restorations were digitally processed by Adobe Photoshop software, separated into the following four groups and assessed by two calibrated examiners: Group A: The original photograph displayed at 100%, without modifications (IMG100); Group B: Formed by images enlarged at 150% (IMG150); Group C: Formed by digital photographs displayed at 100% (mIMG100), with digital modifications (levels adjustment, shadow and highlight correction, color balance, unsharp Mask); and Group D: Formed by enlarged photographs displayed at

150% with modifications (mIMG150), with the same adjustments made to Group C. Photographs were assessed on a calibrated screen (Macbook) by two calibrated clinicians, and the results were statistically analyzed using Wilcoxon tests (SSPS 11.5) at 95% CI. Results: The photographic method produced higher reliability levels than the direct clinical method in all parameters. The evaluation of digital images is more consistent with clinical assessment when restorations present some moderate defect (Bravo) and less consistent when restorations are clinically classified as either satisfactory (Alpha) or in cases of severe defects (Charlie). Conclusion: The digital photographic method is a useful tool for assessing the quality of dental restorations, providing information that goes unnoticed with the visual-tactile clinical examination method. Additionally, when analyzing restorations using the Ryge modified criteria, the digital photographic method reveals a significant increase of defects compared to those clinically observed with the naked eye. Photography by itself, without the need for enlargement or correction, provides more information than clinical examination and can lead to unnecessary overtreatment.

INTRODUCTION

The use of digital photography is becoming a standard for today's modern dental practices[1] through the photographic documentation of clinical findings prior to initiating restorative treatment. Digital intraoral photography has greatly influenced the ease of documentation and the storage of clinical images of specific clinical situations. As a result, its use in dentistry is consistently increasing.[2-5]

Some of the uses of digital photography include evaluation of restorations,[6,7] color selection of composite resins[8], control of tooth whitening,[9] and evaluation of tooth wear.[10] It has also been used to measure the color of healthy gingiva[11] and for recording and analysis in orthodontics therapy.[2] Secondary uses include dento-legal documentation, education, communication, portfolios, and marketing.[12]

Additionally, because digital photography possesses many features that can improve the practice of dentistry,[2,13,14] including the ability for the clinician to edit images using software programs,[5,9,11,15,16] this technology could also be considered as an indirect method of detection, especially in the assessment of restorations, because direct evaluation alone has proven to be insufficient in identifying early changes in the development of defects on restorations.[17-20]

Although digital photography presents interesting features for indirect diagnosis, its correlation to clinical detection is still not clear. Moreover, the use of photography, along with the manipulation of images with Adobe Photoshop software—the ability to adjust an image to its intended brightness, contrast, and color without misrepresenting the original image and treatment outcome—has not yet been described in the field of operative dentistry.

The aim of this study was to compare the efficacy of direct clinical evaluation with indirect digital photographic assessment of amalgam and resin-based dental restorations. The research null hypothesis of this study was that direct clinical and indirect photographic assessment of the quality of amalgam and resin-based composite restorations presented similar performance.

MATERIALS AND METHODS

This study was conducted in permanent teeth of Caucasian adult patients in the city of Santiago, Chile. Approval and ethical permission were obtained from the Ethics Committee in the Dentistry Research Office of the Dental School at Chile University (UChile PRI-ODO-0207). The sample consisted of 89 restorations from 36 patients attending the Control Clinic of Operative Dentistry (maintenance) at the University of Chile. On arrival at the clinic, the purpose of the research and the procedures of the study were explained in detail to the patients, consent was requested for the photography and for a standard dental exam, and patients who accepted the conditions of the study signed an informed consent form.

Seven parameters, including color (only for resin-based restorations), occlusal marginal adaptation, anatomical form, roughness, occlusal marginal stain, luster, and secondary caries, were assessed in 89 Class I and Class II restorations (32 composite and 57 amalgam) from 36 adults using the modified US Public Health Service (USHPS)/Ryge criteria (Table 1). Inclusion criteria consisted of adult patients in good hygienic condition with Class I and Class II amalgam or resin-based composite restorations.

The clinical detection of secondary caries (Charlie) was made according to Ekstrand's criteria.[21] The photographic secondary caries detection criteria

Table 1: *Modified Ryge/USPHS Clinical Criteria (N/A = Not Applicable)*

Clinical Characteristics	Alpha	Bravo	Charlie
Color	The restoration matches in color and translucency to adjacent tooth structure	The mismatch in color and translucency is within the acceptable range of tooth color and translucency	The mismatch is outside the acceptable range of color and translucency
Marginal adaptation	Explorer does not catch or has one-way catch when drawn across the restoration/tooth interface	Explorer falls into crevice when drawn across the restoration/tooth interface	Dentin or base is exposed along the margin
Anatomic form	The general contour of the restoration follows the contour of the tooth	The general contour of the restoration does not follow the contour of the tooth	The restoration has an overhang
Surface roughness	The surface of the restoration has no surface defects	The surface of the restoration has minimal surface defects	The surface of the restoration has severe surface defects
Marginal staining	There is no discoloration between the restoration and tooth	There is discoloration on less than half of the circumferential margin	There is discoloration on more than half the circumferential margin
Secondary caries	There is no clinical diagnosis of caries	Not applicable	There is clinical diagnosis of caries
Luster of restoration	The restoration surface is shiny and has an enamel-like, translucent surface	The restoration surface is dull and somewhat opaque	The restoration surface is distinctly dull and opaque and is esthetically displeasing

were based on surface staining, surface irregularities, and loss of dental tissue in the margins of the restorations.

Direct intraoral clinical examination was carried out by two calibrated examiners (Cohen's Kappa 0.76). Each restoration was clinically examined independently at the beginning of the study for the parameters of color, marginal adaptation, anatomic form, surface roughness, occlusal marginal stain, luster, and secondary caries. The quality of the restorations was evaluated according to USPHS/Ryge criteria (Table 1), which states the use of an eye without any magnification device, only a dental mirror and an explorer, in a proper isolated field following the directions to assess every parameter.[22] If any difference was found between both examiners, a third calibrated examiner (Cohen's Kappa 0.76) established the final diagnosis.

Teeth were examined after drying with the air of a triple syringe, using the artificial light of the dental unit (Forest Dental Products Inc, Hillsboro, OR, USA). The instruments used for the exam were plain number 5 ss mirrors (Zirc Dental Products, 3918 Highway 55, Buffalo, MN, USA), explorer no. 54 SE (Hu Friedy, Chicago, IL, USA), and tongue depressors (Henry Shein Inc, Melville, NY, USA).

Photographic Method

Standardized photographs were taken of each restoration on the same day of the clinical exam using a digital single-lens reflex camera (Nikon-D100, Tokyo, Japan) with a 105-mm Microlens (AF-S 1:2.8 VR Nikkor Nikon G) and with a Macro Speed flash SB-29s (Nikon Inc, Melville, NY, USA). The quality of the photos was set on JPEG fine and 12.0 megapixels. Camera settings included manual operation mode, ISO 400, F-8, speed 80, color space RGB.

Photographs were taken by an expert clinical photographer, with the patients sitting on a dental chair and leaning back to avoid movements during focusing and photography. An assistant provided retraction of the cheek and lips. Saliva and food fragments were removed with air or sterilized gauze when necessary. Pictures were taken by focusing on the center of the restorations. The camera was placed perpendicular to the occlusal surface or tilted no more than 20° to the tooth plane to minimize mirror reflection and burnout of the picture. Each photograph was evaluated for acceptability and quality; if it was not acceptable, the photograph was repeated.

Pictures were saved on an Apple MacBook laptop MC516CI/A (Apple Inc, Cupertino, CA, USA), which was calibrated using the spectrophotometer Efi es-1000 (EFS Inc, Foster City, CA, USA). Subsequently, the pictures were randomly edited using the software Adobe Photoshop CS3 Extended v10.0 (Adobe Systems Inc, San Jose, CA, USA), creating four groups of images from each original photograph:

Group A: Formed by the original photograph displayed at 100% (IMG100), without modifications;

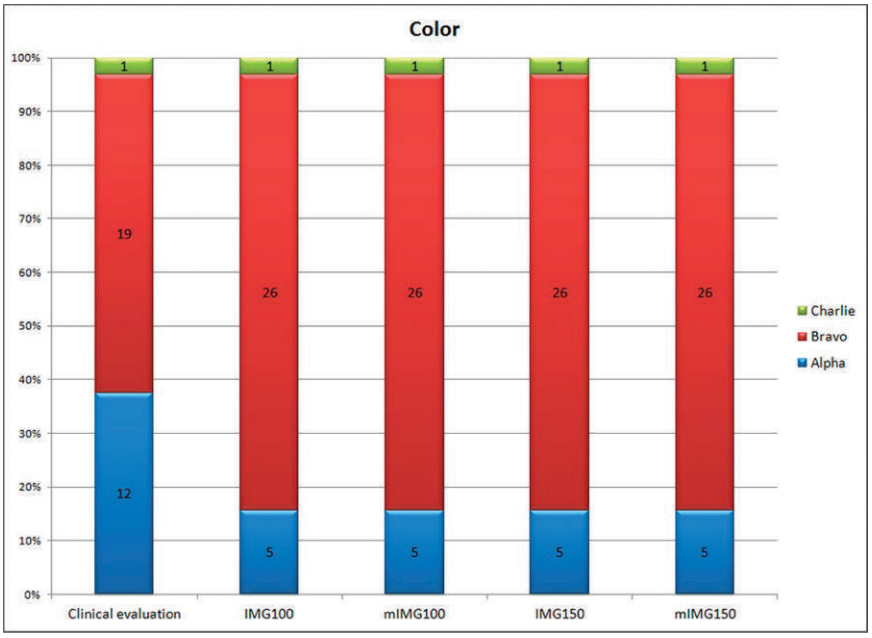

Figure 1. *Color observations separated by groups and quality evaluation expressed as a percentage of USPHS/Ryge criteria.*

the photograph was cropped to leave the teeth and the restoration centered in the picture, using the following specifications: 866 × 630 pixels at a resolution of 100 pixels per inch (Figure 1).

Group B: Formed by images enlarged at 150% (IMG150), using the free transform tool from the editing command, without modifications (Figure 2).

Group C: Formed by digital photographs displayed at 100% (mIMG100), with digital modifications that included level adjustments, shadow and highlight correction, color balance, and unsharp mask (Table 2; Figure 3).

Group D: Formed by enlarged photographs displayed at 150% (mIMG150) with modifications; the same adjustments that were made to Group C were made in this group (Figure 4).

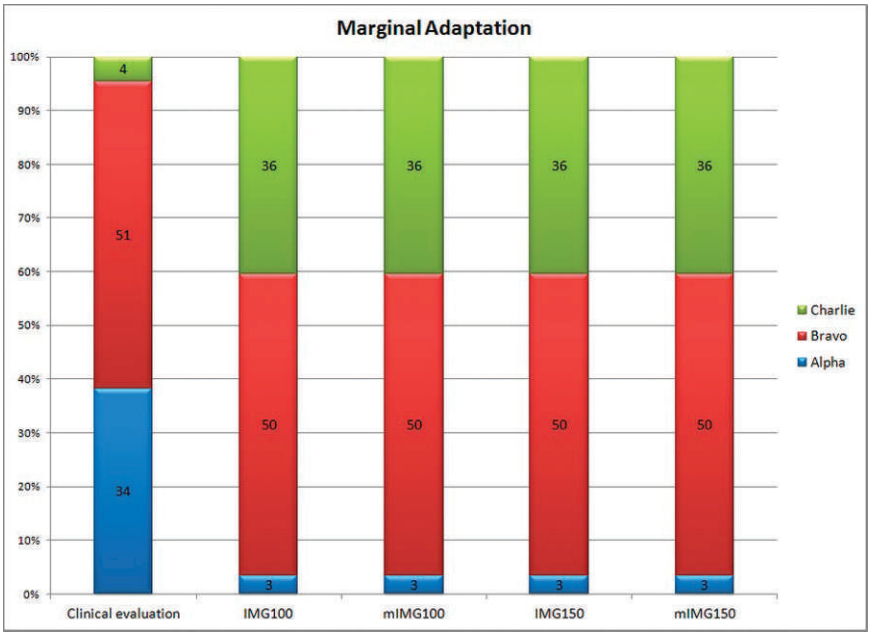

Figure 2. *Marginal adaptation observations separated by groups and quality evaluation expressed as a percentage of USPHS/Ryge criteria.*

Table 2: *Digital Parameters Corrections Applied to Photograph of Groups C and D*

	Shadow Adjustment	Highlights Adjustment
Amount	50% ± 10%	Between 0% and 10%
Tonal width	50%	50%
Radius	30 pixels	30 pixels

Photographs in Groups C and D were obtained by the digital manipulation of IMG100 and IMG150 using the following four tools:

Levels Adjustment: Correction of tonal distribution of each photograph, using the command Image/adjustments/levels in the red, green, and blue channel, adjusting the histogram and bringing the image to a normal or Gaussian distribution.

Shadows, Highlights Adjustment: These were corrected separately without affecting the midtones optimized in the previous step. All images were stored in JPEG format.

Color Balance: The command image/adjustments/color balance was used to approximate the natural color image of the photographed structures using the gingival color as a reference.

Unsharp Mask Filter: To enhance the detail of important areas of the images, the unsharp mask filter was applied with the following values: amount: 100%-–170%; radius: 1.6 ± 0.5; threshold: 0.

Each photograph was assessed and scored independently by two calibrated examiners (Kappa = 0.76) with the same criteria used in the clinical method. Disagreements between examiners were solved by a similar system used in clinical detection.

Data Analysis

The results of all assessments (clinical, IMG100, mIMG100, IMG150, and mMG150) were compared to the differences detected using the nonparametric Friedman test. Additionally, to determine whether the enlargement of the image influenced the results of the evaluation, images at 100% (IMG100 and IMG100m) were compared to images at 150% (IMG150 and IMG150m). Furthermore, to evaluate the influence of image manipulation, edited images (mIMG150 and mIMG100) were compared to non-edited (IMG100, IMG150) images using the non-parametric Wilcoxon test. In all tests, the level of confidence was set at $p = 0.05$, and calculations were performed using the SSPS 11.5 software package (SPSS Inc, Chicago, IL, USA).

RESULTS

Seven parameters were evaluated with clinical photographic methods in 36 patients (mean age 26.7 years) with 89 posterior dental restorations, both Class I (n=51) and Class II (n=38); (32 composite and 57 amalgam). Only composite-based resin restorations were evaluated for color.

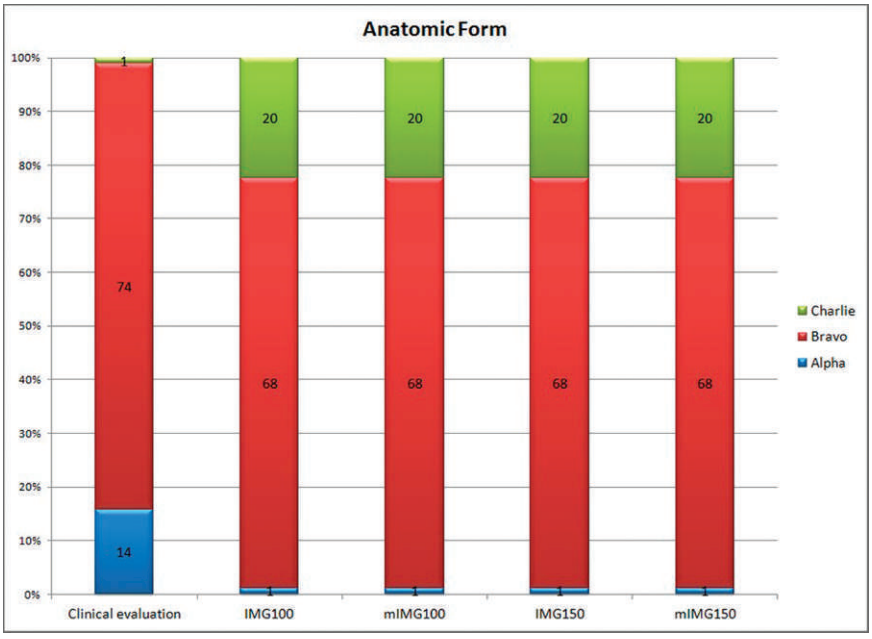

Figure 3. *Anatomic form observations separated by groups and quality evaluation expressed as a percentage of USPHS/Ryge criteria.*

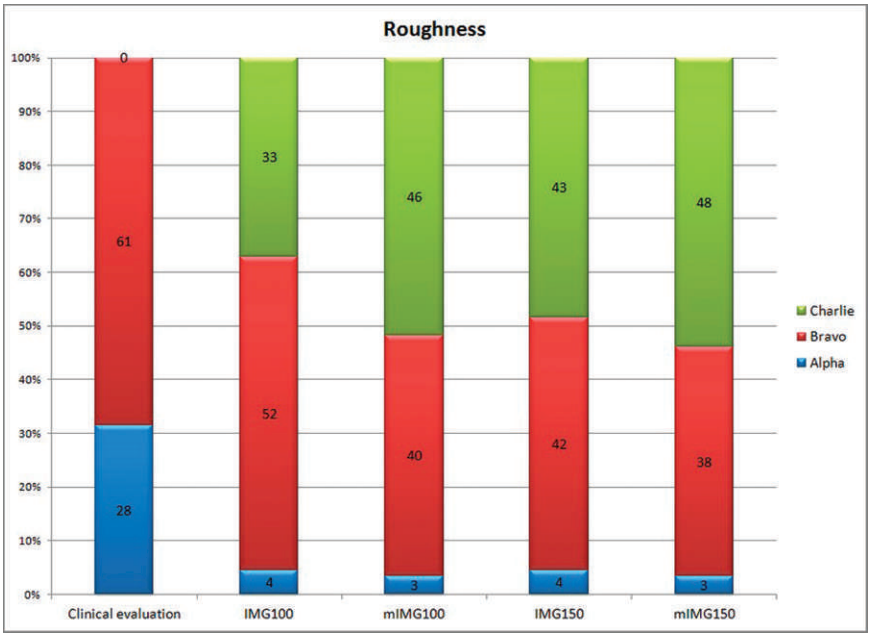

Figure 4. *Surface roughness observations separated by groups and quality evaluation expressed as a percentage of USPHS/Ryge criteria.*

In general, the present study revealed a moderate agreement between clinical and photographic assessment methods for dental restorations.

The evaluation of digital images appeared to be more consistent with clinical assessment when the restorations were in acceptable condition with one or more defective parameters (Bravo), but the results of these methods were less consistent when the restorations were clinically classified to be in excellent condition (Alpha) or in cases of severely deficient restorations (Charlie).

In the evaluation of restoration color, the resin-based composite restorations were judged to be more acceptable when they were clinically evaluated ($p<0.05$). There were no statistically significant differences among the four groups of images (Figure 1).

Image examination resulted in a greater number of restorations that were judged as Charlie and fewer Alpha values when compared to clinical examination for the parameters marginal adaptation, anatomic form, and marginal staining ($p<0.05$). Among the groups of images, there was no difference observed when either enlargement or manipulation was applied (Figures 2, 3, and 5).

When roughness was evaluated, the image evaluation presented an increase of observed Charlie values and a decrease in Alpha and Bravo values when compared to the results of clinical examination ($p<0.05$). Among the groups of images, IMG150, IMG100m, and IMG150m revealed more restorations that were assessed as Charlie and fewer that were judged as Bravo than the IMG100 group ($p<0.05$). When comparing the five groups all together, the restorations evaluated on the images were considered to be more degraded than their clinical counterparts ($p<0.05$; Figure 4).

Luster assessment showed a similar trend; that is, restorations were judged to be in worse condition when images were evaluated ($p<0.05$). They showed an increase in Bravo and a decrease in Alpha values when compared to clinical examination ($p<0.05$). Among the groups of images, there was no difference when either enlargement or manipulation was analyzed (Figure 6). Some examples of photographic evaluation are included in Table 3.

When photographs were used to detect secondary caries, in all groups, an increase in the number of reported Charlie values was observed relative to the results obtained by direct clinical detection in which no caries lesions were detected. Statistically significant differences were observed for groups IMG100m, IMG150, and IMG150m ($p<0.05$). Those patients were clinically examined again, and no caries lesions were clinically observed.

DISCUSSION

Dental photography is a simple and inexpensive imaging method that does not involve ionizing radiation or discomfort. The use of photographs to

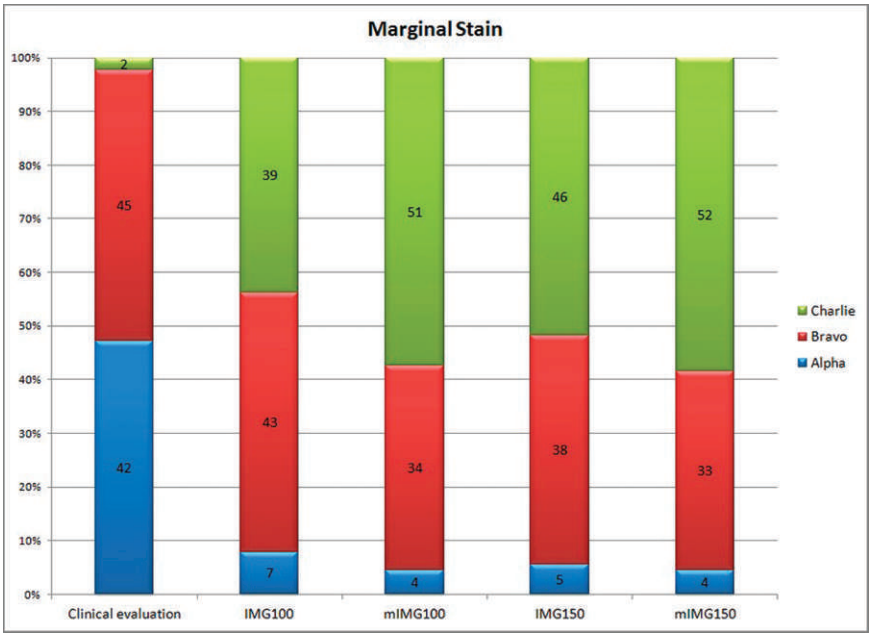

Figure 5. *Marginal stain observations separated by groups and quality evaluation expressed as a percentage of USPHS/Ryge criteria.*

evaluate restorations is based on the belief that by standardizing the gathering and the processing of photographs, it would be possible to develop a reliable method suitable for use in operative dentistry. Although photography is not used routinely as a method of restoration evaluation, it appears to be a promising control and diagnostic tool in operative dentistry treatments.

Some of the important advantages of using photographic images as an indirect detection method include the fact that it allows for more evaluation time, in stable conditions, which is not always possible in a direct clinical examination.[15] Furthermore, well-composed images that are reviewed on a large monitor away from the treatment room's extraoral and intraoral distractions can ensure that

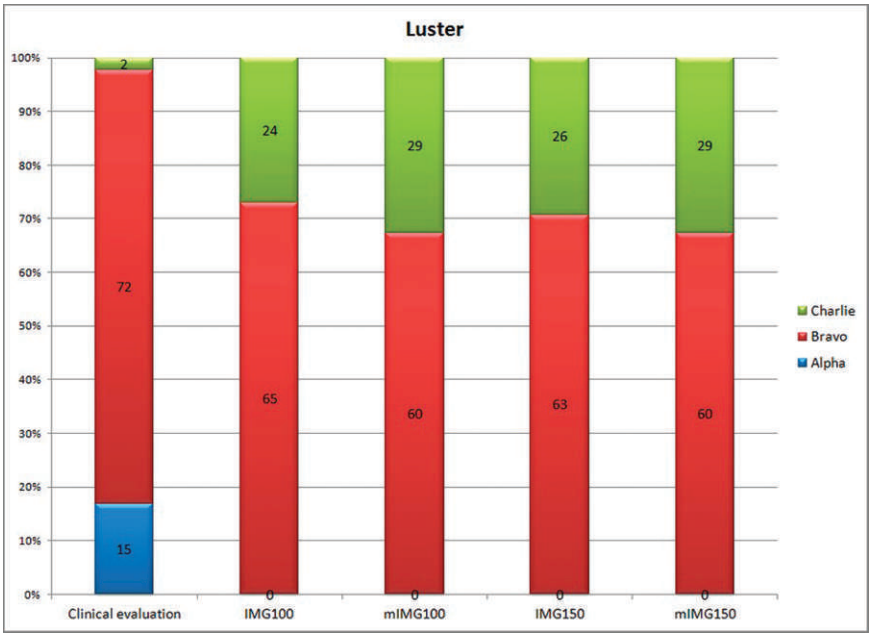

Figure 6. *Luster observations separated by groups and quality evaluation expressed as a percentage of USPHS/Ryge criteria.*

Table 3: *Images Evaluation, by USPHS/Ryge Criteria, of Amalgam and Resin-Based Composite Restorations, Separated by Parameters (N/A = Not Applicable)*

	Amalgam Restorations					Composite Restorations				
	Figure 8 (Group A)	Figure 9 (Group B)	Figure 10 (Group C)	Figure 11 (Group D)	Clinical Evaluation	Figure 12 (Group A)	Figure 13 (Group B)	Figure 14 (Group C)	Figure 15 (Group D)	Clinical Evaluation
Color	N/A	N/A	N/A	N/A	N/A	Alpha	Bravo	Bravo	Bravo	Alpha
Marginal adaptation	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo
Anatomic form	Alpha	Bravo	Alpha	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo
Surface roughness	Bravo	Charlie	Charlie	Charlie	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo
Marginal stain	Bravo	Bravo	Charlie	Charlie	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo
Luster	Bravo	Charlie	Bravo	Charlie	Bravo	Bravo	Bravo	Bravo	Bravo	Bravo
Secondary caries	Alpha	Alpha	Alpha	Alpha	Alpha	Alpha	Alpha	Alpha	Alpha	Alpha

an accurate diagnosis is formulated.[8] Other studies that focused on the detection of developing enamel defects have shown the same trend observed in the current study, concluding that photographic methods were more sensitive than direct clinical examination in permanent teeth.[23]

In addition, the advent of image editing software, such as Adobe Photoshop, has made it possible to manipulate images to either correct or enhance them. This study applied both enlargement and photo correction. Enlargement is performed by interpolation using algorithms to obtain a larger image, whereas correction is applied to bring an image back to its intended brightness, contrast, and color.

Concerning enlargement, it might be claimed that this process can cause image deterioration, altering the perceived status of the restoration. However, this concern is alleviated by capturing images with a high quality and quantity of pixels, recording as much detail as possible at the outset. This way, enlargement can be considered to be a valuable tool for assessing the status of restorations over time at a size that is larger than the real object, revealing

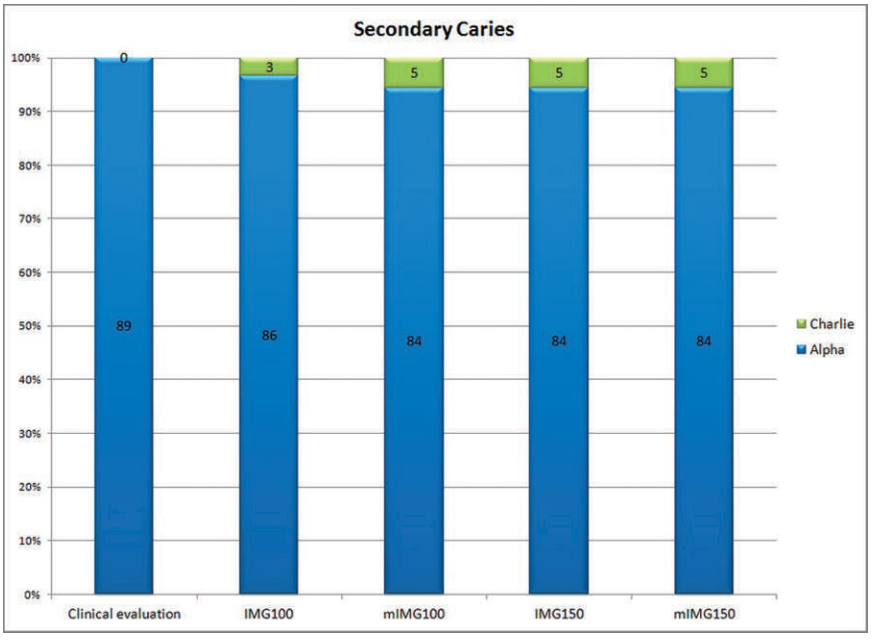

Figure 7. *Secondary caries observations separated by groups and quality evaluation expressed as a percentage of USPHS/Ryge criteria.*

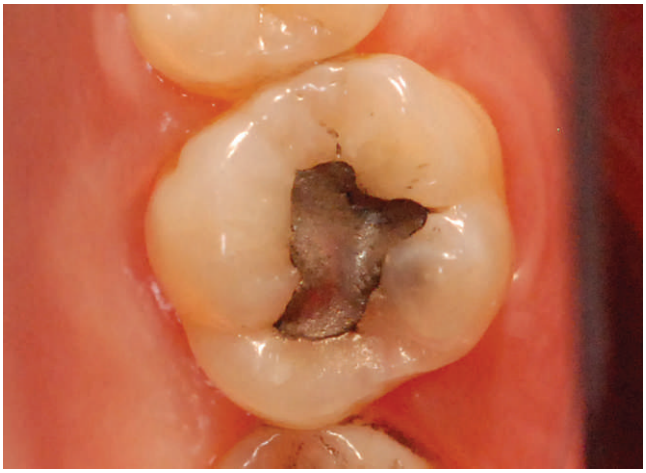

Figure 8. *Group A: The original photograph of amalgam restoration displayed at 100%, without modifications (IMG100).*

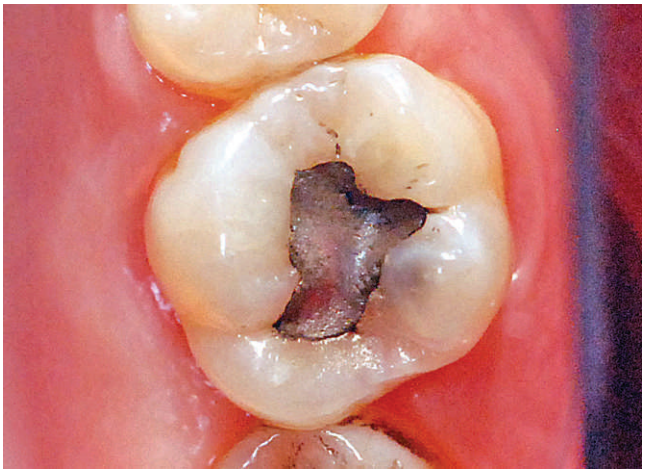

Figure 10. *Group C: Digital photographs of amalgam restoration displayed at 100% (mIMG100), with digital modifications.*

information that usually goes unnoticed.[15] In fact, in this study, enlargement increased the number of restorations that were judged to be unsatisfactory when compared to the results of clinical evaluation.

With regard to photo correction, it is important to remember that dental images are dento-legal documents. Therefore, manipulation should be kept to a minimum, ensuring that the original image is not altered to an extent that it hides pathology or alters the clinical situation to camouflage what was present in the oral cavity.[15] In the current study, correction did not alter the results of evaluation when compared to the original image (IMG100), except for the parameters of roughness and secondary caries. In other words, an image of good quality at 100% of its pixels would be enough to evaluate the quality of restorations. Nevertheless, it must be noted that a picture is a two-dimensional representation of a three-dimensional structure; therefore, photos should be used only in an indirect or complementary evaluation method.[24]

The results of this study indicate that more problems were detected in restorations when they were evaluated by means of images than by clinical examination, agreeing with the results of the study by Smales;[6] thus, the use of digital imaging resulted in a significant increase in the number of restorations that received Bravo and Charlie values. These results suggest that the clinician should consider the differences between both methods of evaluation and relate them to treatment decisions.

Regrettably, no previous study has compared these two methods of assessing the quality of dental

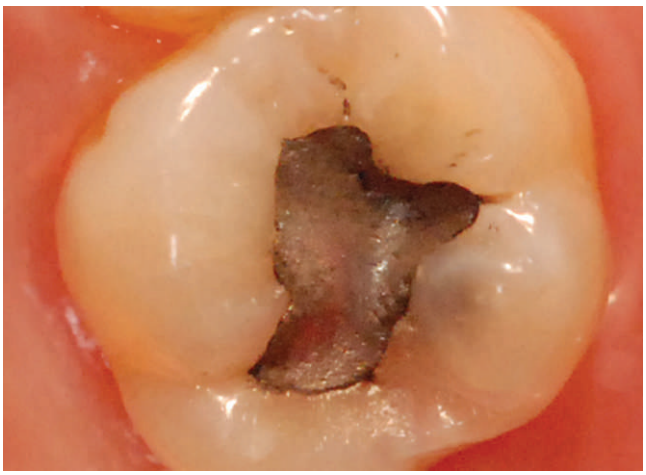

Figure 9. *Group B: Images of amalgam restoration enlarged at 150% (IMG150).*

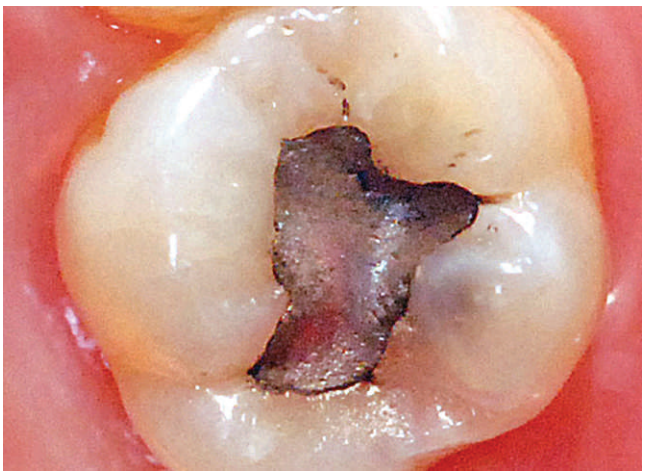

Figure 11. *Group D: Enlarged photographs of amalgam restoration displayed at 150%, with modifications (mIMG150).*

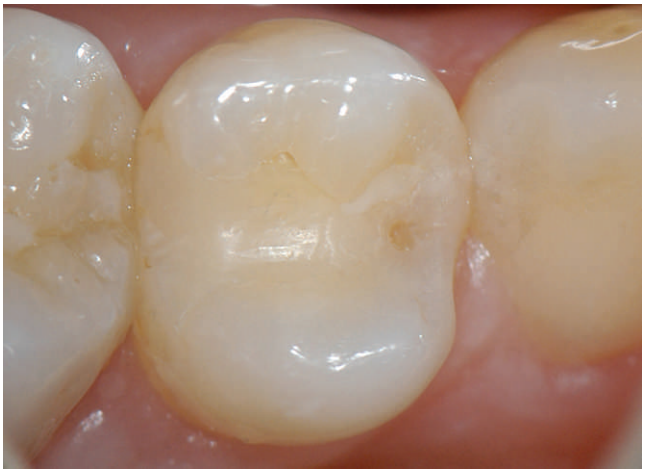

Figure 12. *Group A: The original photograph of resin-based composite restoration, displayed at 100%, without modifications (IMG100).*

restorations. The present study used a powerful digital camera, well equipped with accessories and settings that allowed the photographer to easily zoom and focus to obtain the best possible pictures of the restorations. These images allowed the examiners to view the photographs at different conditions without the technical problems that might be encountered when using nondigital photos. Additionally, the photographic method provided permanent records of the restorations and the teeth, with less bias than other methods; photography also accelerated the time of the clinical exam, as it did not include laboratory processing, and there was no need to consider the possibility of cross infection.[25]

When evaluating color, the teeth were subjected to different lighting conditions: the flash of the camera

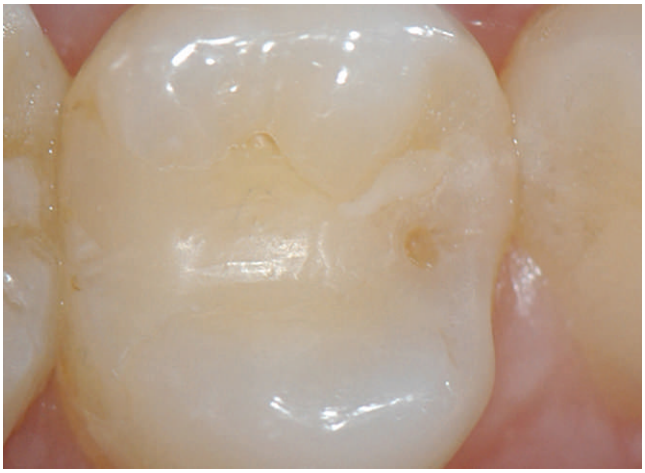

Figure 13. *Group B: Images of resin-based composite restoration, enlarged at 150% (IMG150).*

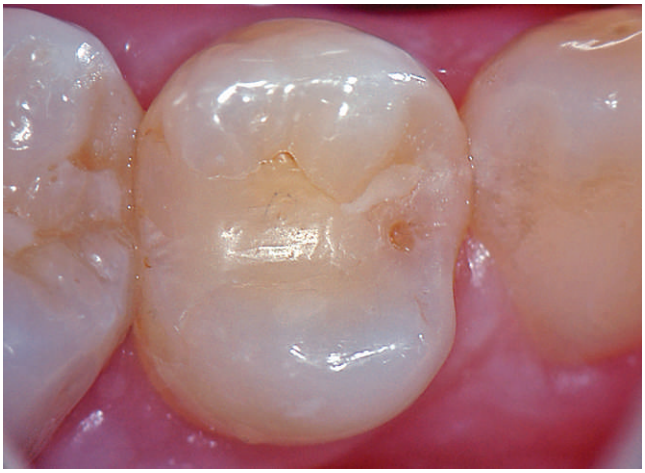

Figure 14. *Group C: Digital photographs of resin-based composite restoration, displayed at 100% (mIMG100), with digital modifications.*

during the photography and the light source of the dental unit in the clinic, which may generate the phenomenon of metamerism.[26,27] Also, when marginal adaptation was evaluated, the clinical approach has the advantage of probing with an explorer in addition to visual assessment.

Concerning luster, the restorations were judged to be duller (matte) when assessed photographically; in fact, in this evaluation, there were no restorations that received Alpha values for this parameter. According to Ahmad,[28] this may be due to the use of a circular flash unit, which has a uniform light output, creating an image devoid of shadows, which appears flat, smooth, and dull.

Importantly, through the evaluation of images without amplification (IMG 100), some defects that

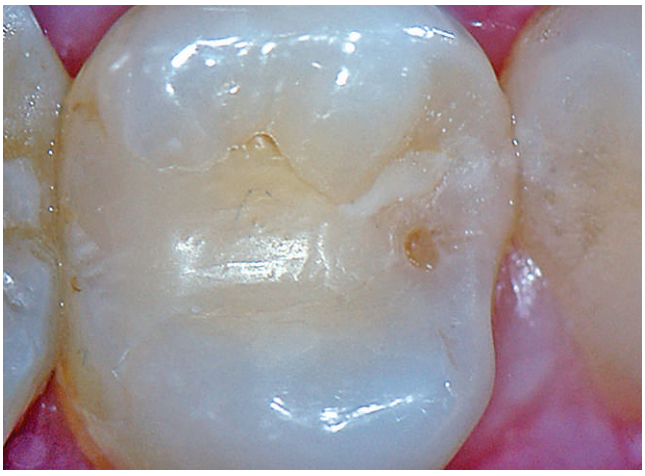

Figure 15. *Group D: Enlarged photographs of resin-based composite restoration, displayed at 150%, with modifications (mIMG150).*

went unnoticed clinically were detected, especially for the parameters of marginal adaptation, anatomic form, roughness, and staining of margins. However, it is not possible to establish whether this situation corresponds to overdetection or whether it constitutes evidence that the evaluation of images definitely allows for the detection of defects that can remain unseen clinically, in this way revealing the limitations of clinical evaluation.

The comparison between groups for the evaluation of secondary caries demonstrated that all photographic groups showed a significant overdetection compared with clinical detection. Additionally, in the photographic groups, there were no observed differences between the magnified, modified, or unaltered pictures. In light of these results, patients were clinically examined again, and marginal caries lesions were not detected. This discrepancy is significant, as it suggests that photographic methods may promote unnecessary dental overtreatment, especially in populations with low caries risk. It must be stressed that photographic detection of secondary caries presents a huge disadvantage, as it is not possible to probe dental tissue hardness; therefore, it provides only limited information for this parameter.

For many years, professionals in the field of operative dentistry have known that dental restorations present a limited range of life, representing an important concern for the patients, institutions and clinicians involved. The early detection of localized restoration defects could facilitate the repair of such restorations instead of replacement.[29-32] "Using photographs as a way to store visual information after finishing dental restorations can help the clinician for monitoring its ageing throughout time. This method allows for implementing proper maintenance measures to improve restoration longevity.[33,34] It is useful for determining the mean life of restorations and for providing basic information for long-term studies and teaching.

Examiners of the current study reported that photography, as a complementary exam tool, provides additional information when they were in doubt, allowing them to make better decisions.

CONCLUSIONS

Digital photography is a useful tool for assessing the status of restorations, providing information that goes unnoticed with the visual-tactile clinical examination method. But this information can lead to unnecessary overtreatment.

When analyzing restorations using the Ryge modified criteria, the digital photography method reveals a significant increase in the number of detected defects. The digital photography method by itself, without the need for enlargement or correction, provides more information than clinical examination.

Finally, the evaluation of digital images is more consistent with clinical assessment when the restorations have some moderate defect (Bravo) and are less consistent when the restorations are clinically classified as either satisfactory (Alpha) or in cases of severely deficient restorations (Charlie). This is the first study that provides information of the comparison between clinical assessment and photographic evaluation of dental restorations under USPHS/ Ryge modified criteria.

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.
Study ascribed to research project UChile-PRI-ODO-0207.

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Measurement of the Internal Adaptation of Resin Composites Using Micro-CT and Its Correlation With Polymerization Shrinkage

HJ Kim • SH Park

Clinical Relevance

Resin composites with low levels of polymerization shrinkage strain and stress resulted in better internal adaptation. This effect may be related to the low incidence of complications, such as postoperative hypersensitivity.

SUMMARY

In the present study, the internal adaptation of dentin-composite interfaces with various resin composite materials under conditions of thermomechanical loading was analyzed nondestructively using micro–computed tomography (micro-CT), and these results were compared with analyses of microgaps after sectioning. Additionally, the correlation of internal adaptation with polymerization shrinkage strain and stress was evaluated.

Four nonflowable resins, Gradia Direct (GD), Filtek P90 (P9), Filtek Z350 (Z3), and Charisma (CH), and two flowable resins, SDR (SD) and Tetric N-Flow (TF) were used. First, the polymerization shrinkage strain and stress were measured. Then, Class I cavities were prepared in 48 premolars. They were divided randomly into six groups, and the cavities were filled with composites using XP bond. To evaluate the internal adaptation, tooth specimens were immersed in a 25% silver nitrate solution, and micro-CT analysis was performed before and after thermomechanical loading. The silver nitrate penetration (%SP) was measured. After buccolingual sectioning and rhodamine penetration of the specimen, the rhodamine penetration (%RP) was measured using a stereomicroscope. One-way analysis of variance was then used to compare the polymerization shrinkage strain, stress, %SP, and %RP among the groups at a 95% confidence level. A paired *t*-test was used to compare the %SP before and after thermomechanical loading. Pearson correlation analysis was used to compare the correlation between polymerization shrinkage strain/stress and %SP or %RP to a 95% confidence level.

Evaluation of the polymerization shrinkage strain demonstrated that P9 < Z3 ≤ GD < CH

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≤ SD < TF ($p<0.05$); similarly, evaluation of the polymerization shrinkage stress showed that P9 ≤ GD ≤ Z3 ≤ CH ≤ SD < TF ($p<0.05$). The %SP showed that P9 ≤ GD ≤ Z3 < CH ≤ SD < TF ($p<0.05$) before loading and that P9 ≤ GD ≤ Z3 ≤ CH ≤ SD < TF ($p<0.05$) after loading. There was a significant difference between the before-loading and after-loading measurements in all groups ($p<0.05$). Additionally, there was a positive correlation between the %SP and the %RP ($r=0.810$, $p<0.001$).

Conclusively, the polymerization shrinkage stress and strain were found to be closely related to the internal adaptation of the resin composite restorations. The newly proposed model for the evaluation of internal adaptation using micro-CT and silver nitrate may provide a new measurement for evaluating the internal adaptation of restorations in a nondestructive way.

INTRODUCTION

Resin composites are generally used as restorative materials because of their good esthetics and ability to adhere to tooth structure using adhesive. However, the conversion of the resin composite monomers into a polymer network is accompanied by a bulk contraction leading to 1.67%-5.68% volumetric polymerization shrinkage.[1] Polymerization shrinkage generates stress at the tooth-restoration interface and may lead to microgap formation and microleakage, the latter of which allows for the infiltration of saliva and bacteria. This infiltration can lead to secondary caries, pathologic pulpal changes, and restoration failures.[2]

Souza-Junior and others[3] evaluated the internal adaptation of restorations by sectioning the samples. In their study, internal gaps formed prominently at the pulpal and axio-pulpal line angles of the restorations. The gap formation could cause fluid flow in the dentin tubules, and the transduction of dentinal fluid through the dentin adhesive could produce dentinal fluid-filled regions, which could contribute to the degeneration of adhesives.[4] The internal adaptation of dentin-restoration interfaces has generally been evaluated by dye penetration with basic fuchsin, methylene blue, erythrosin, silver nitrate, or radioactive markers and by sectioning the samples.[5] Although very popular, the dye penetration method exhibits inherent limitations in that the type, size, and concentration of the tracer, the pH of the aqueous immersion solutions, and the chemical affinity of the tracer with hard dental tissues all influence the results obtained.[6] Furthermore, sectioning destroys the sample and renders additional testing impossible, and gaps measured in a selective area cannot represent the entire sample.[7]

For these reasons, direct imaging techniques with micro–computed tomography (micro-CT) are becoming more widely used.[8] Micro-CT obtains the three-dimensional (3D) structures of small objects with a high level of spatial resolution. In dental research, micro-CT imaging has been used to analyze the structures at dentin-adhesive-composite interfaces before and after mechanical loading[9] and to evaluate resin composite volume and 3D marginal adaptation before and after polymerization.[6,10] However, such studies did not use dentin adhesives, and their clinical relevance was therefore very low. Kwon and Park[11] evaluated the internal adaptation of adhesive restorations with and without a resin-modified glass ionomer base using micro-CT analysis of human molars. Using micro-CT and the silver nitrate infiltration technique through the dentinal tubules of the pulpal side, the dentin-composite resin interface was evaluated nondestructively.

It is challenging to develop restorative materials that do not produce microgaps, and the current research on new materials is insufficient. To evaluate restorative materials, further research about internal adaptation and microleakage will therefore be very important.

The aim of this study was to evaluate the internal adaptation of resin composites using the nondestructive technique of micro-CT, to compare these results with the microgaps found in histologic sections, and to evaluate their correlation with polymerization shrinkage.

The null hypotheses are as follows.

1) No differences exist in the internal adaptation among the resin composites tested with micro-CT.

2) No correlation exists between polymerization shrinkage stress/strain and internal adaptation tested with micro-CT.

3)No correlation exists between the internal adaptation tested with micro-CT and the microgaps evaluated with microscopy and the dye solution.

4) No correlation exists between the polymerization shrinkage stress/strain and the microgaps evaluated with microscopy and the dye solution.

METHODS AND MATERIALS

This study was approved by the local ethics committee (IRB 2-2012-0060).

Table 1: *Composite Materials Used in This Study*[a]

Code	Product	Manufacturer	Base Resin	Filler, wt%/vol%	EM, GPa
P9	Filtek P90	3M ESPE, St Paul, MN, USA	Silorane-based	76%/55%	9.6
GD	Gradia Direct	GC Co, Milford, DE, USA	UDMA dimethacrylate co-monomers	73%/64%	6.3
Z3	Filtek Z350	3M ESPE, St Paul, MN, USA	Bis-GMA/EMA, UDMA	78.5%/59.5%	11
CH	Charisma	Heraeus Kulzer, Dormagen, Germany	Bis-GMA, TEGDMA	78%/61%	8
SD	SDR	Dentsply Caulk, Milford, DE, USA	Modified urethane dimethacrylate EBPADMA/TEGDMA	68%/45%	5.7
TF	Tetric N-flow	Ivoclar Vivadent, Schaan, Liechtenstein	Bis-GMA, TEGDMA	63.8%/43%	5.3

Abbreviations: P9, Filtek P90; GD, Gradia Direct; Z3,Filtek Z350; CH, Charisma; SD, SureFil SDR; TF, Tetric N- flow; Bis-GMA, bisphenol A dimethacrylate; Bis-EMA, bisphenol A polyethylene glycol diether dimethacrylate; EBPADMA, ethoxylated bisphenol A dimethacrylate; EM, elastic modulus; TEGDMA: triethyleneglycol dimethacrylate; UDMA, urethane dimethacrylate.

[a] *Base resin composition, filler content and elastic modulus are from manufacturer's technical reports and information.*

Materials

Six different resin composite materials were used. These resin composite materials and their compositions are listed in Table 1.

Polymerization Shrinkage Strain Measurements

Resin composites were transferred to a circular Teflon mold (diameter 4.5 mm, depth 1.3 mm) to ensure that the same volume of resin composite was used for each linometer sample. Next, the materials were transferred to an aluminum disk in a custom-made linometer (R&B Inc, Daejon, Korea) that had previously been coated with a separating glycerin gel, covered with a glass slide, and loaded under constant pressure (Figure 1). The specimens were then light-cured with an LED-type light-curing unit (800 mW/cm^2, Bluephase, Ivoclar Vivadent, Schaan,

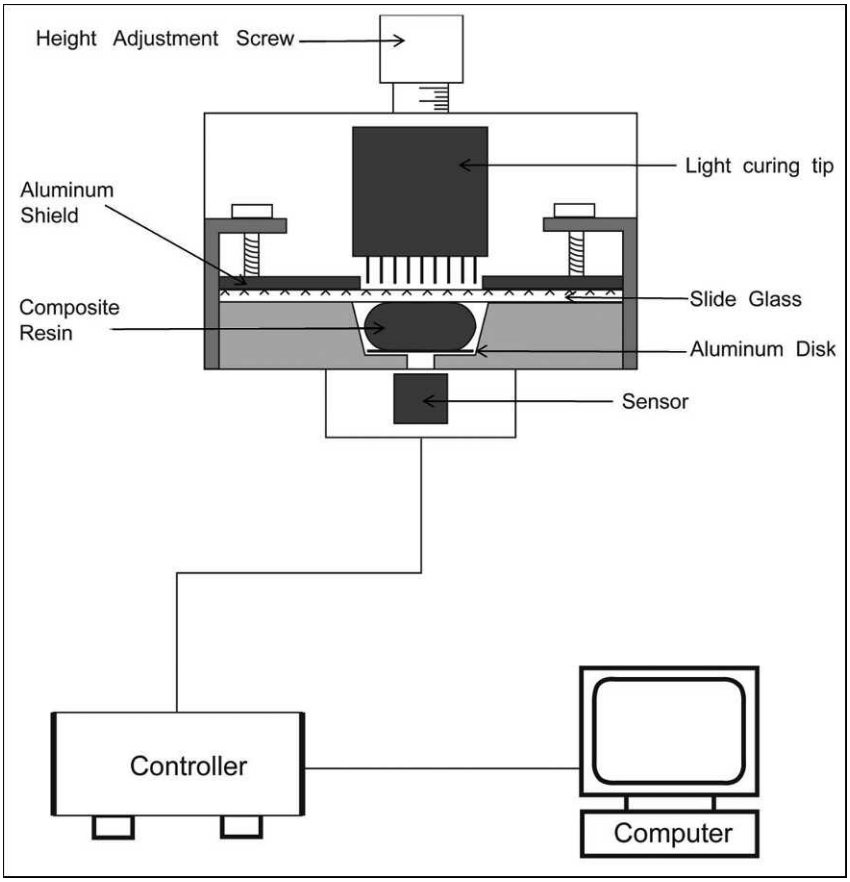

Figure 1. *A schematic diagram of the custom-made linometer.*

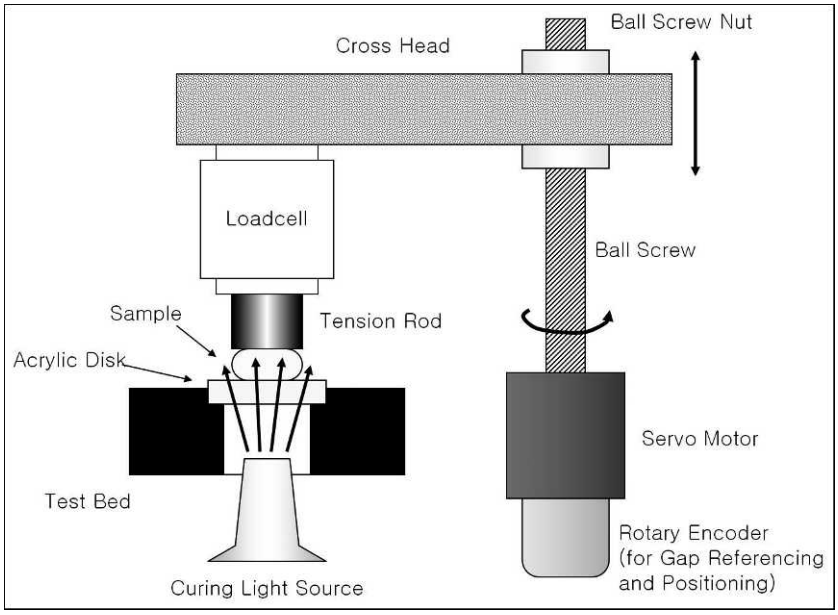

Figure 2. *A schematic drawing of the custom-made polymerization shrinkage stress-measuring machine with a sample in place.*

Liechtenstein) for 40 seconds. The tip of the curing light was positioned 2 mm above the glass slide to ensure the proper curing of the specimens. As the resin composite under the slide glass was cured, it moved the aluminum disk under the resin composite upward. The amount of disk displacement, which was caused by the linear shrinkage of the resin composite, was measured using an eddy current sensor every 0.5 seconds for a period of 120 seconds (Figure 1).

Polymerization Shrinkage Stress Measurements

Polymerization shrinkage stress was measured with a custom-made device and software (R&B Inc) (Figure 2). Resin composite (0.3 g) was transferred to an acrylic disc, and the upper tension rod was set to ensure that the thickness of the specimen was 1 mm (Figure 2). The stress status between the tension rod and the resin composite was set to zero using the software before light curing. Then, the specimens were light-cured with an LED-type light-curing unit (800 mW/cm^2, Bluephase, Ivoclar Vivadent) for 40 seconds through the acrylic disc (Figure 2). At this time, the polymerization shrinkage stress developed, and they were measured by a load cell connected to the tension rod and computer (Figure 2). The software program recorded the polymerization shrinkage stress data simultaneously in the computer every 0.5 seconds for a period of 180 seconds.

Internal Adaptation Measurements

Specimens—Forty-eight intact human premolars extracted for orthodontic treatment were used. In the selecting process, each tooth's dimensions were measured, and the size deviations were controlled within 1 mm. In addition, the thickness of hard tissue, the size of pulp spaces, and the position of pulp horns were evaluated using digital x-ray, and attempts were made to standardize them as far as possible. A high-speed coarse diamond bur (959 KR 314.018, tapered cylinder style with round corner, grit size 100 μm, Komet GEBR Brasseler GmbH & Co KG, Lemgo, Germany) was used to amputate the roots from the cervical regions of the premolars and to prepare occlusal cavities (4 mm mesiodistally, 6 mm buccolingually, 4-mm depth at central portion). The bur was replaced after every eight teeth. Using digital radiographs and the associated software, the distance between the cavity floors and the pulp chambers was controlled to be within 1.0 mm. The 48 specimens were divided randomly into six groups.

The interior parts of the cavities were etched with 10% phosphoric acid (ALL-ETCH, Bisco Inc, Schaumburg IL, USA) for 15 seconds. After irrigation with water for 15 seconds, the cavities were gently dried with an air syringe. For all groups, the dentin adhesive (XP bond, Dentsply Caulk, Milford, DE, USA) was applied for 20 seconds and then air-dried for 5 seconds according to the manufacturer's recommendations. Then, it was cured for 20 seconds

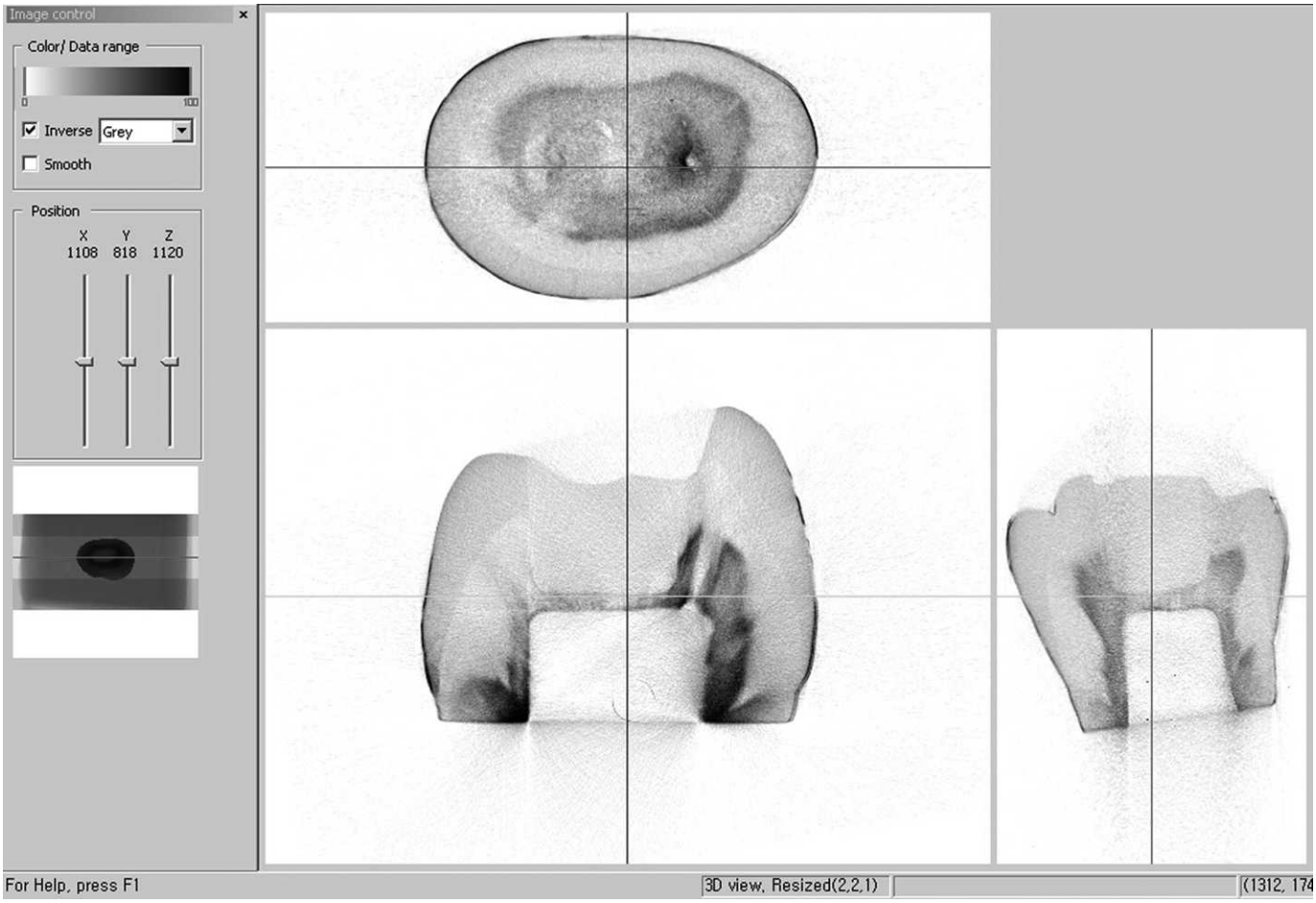

Figure 3. *Reconstruction of the coronal view using the DataViewer program.*

using an LED-type light-curing unit (800 mW/cm^2, Bluephase, Ivoclar Vivadent). The density power of the curing lights was measured using an integration sphere and its software (Gigahertz-Optic GmbH, Puchheim, Germany). For all groups except SDR, the resin composites were applied in two 2-mm increments. For each increment, the resin composites were cured with the LED-type light-curing unit for 20 seconds. SDR was applied as 4 mm of bulk filling and was then cured with the LED-type light-curing unit for 20 seconds, according to the manufacturer's instructions.

Silver Nitrate Solution Application—The pulp chambers were soaked in 17% ethylenediamine tetraacetic acid for 5 minutes and were then washed with saline. The teeth were immersed in a 25% silver nitrate solution under a pressure of 3 kgf for three days.

Thermomechanical Loading With a Chewing Simulator—A chewing simulator CS-4.8 (SD Mechatronik, Feldkirchen-Westerham, Germany) was used to apply a mechanical load of 5 kgf (49 N) 600,000 times under thermodynamic conditions (5°-55°C, dwell time 60 seconds, transfer time 24 seconds). The chewing simulator has eight chambers that simulate vertical and horizontal movements simultaneously under thermodynamic conditions. Each of the chambers consists of an upper sample holder that can fasten the specimen with a screw and a lower plastic sample holder in which the specimen can be embedded.

Micro-CT and Image Analysis—A high-resolution micro-CT (Model 1076, SkyScan, Aartselaar, Belgium) was used to take micrographs under conditions of 100 kV accelerating voltage, a 100 μA beam current, a 0.5 mm Al filter, 18 μm resolution, and 360° rotation at the 0.5° step. Two-dimensional images of 550-560 sagittal and coronal views of each specimen were taken two times (preloading and postloading). During this procedure, each tooth specimen was mounted in a special template that

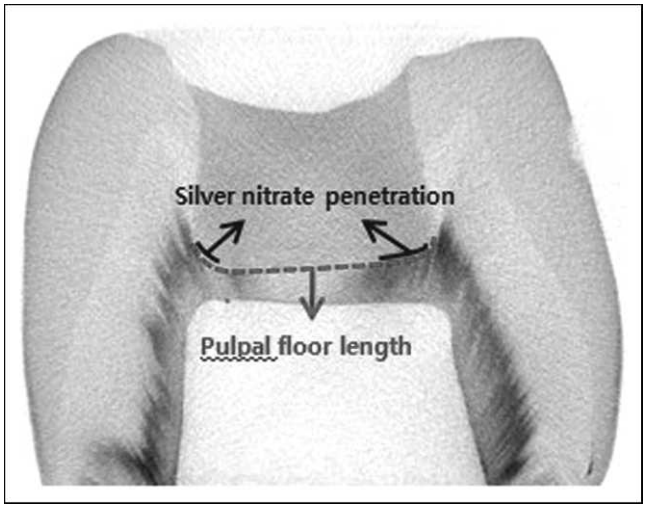

Figure 4. *The %SP calculation method: (silver nitrate penetration length/pulpal wall length) × 100.*

was made specifically for that specimen. This template minimized the change in specimen position during repeated micro-CT imaging. The 2D images were analyzed using the CTAn (SkyScan) and DataViewer (SkyScan) programs. Before image analysis, the density of tooth structure and restoration was measured using the DataViewer (SkyScan) program (dentin: 40-65; resin composite: 90-130; silver nitrate 125-180). The silver penetration into the microgap between the tooth and the restorative materials was considered to be valid when the densities were over the 141 index, which was based on the observation that the areas that were clearly penetrated by the silver nitrate solution had densities >141 on the index when the sagittal and coronal images were compared for the same phase (Figure 3).

Among the 2D images of each specimen, 100 images were selected that clearly confirmed the relationship between the pulpal floors and the silver nitrate. For each specimen, a selection of 2D images was collected before and after mechanical loading; this collection was produced by selecting 100 cuts of 2D images arranged at equal intervals beginning from the central regions of the mesiodistal distances of cavities. The length of the margin of the pulpal floor with a microgap or intact margin was calculated for each image of each specimen, and all the data were then collected and summed. The ratio of the silver nitrate penetration length into the microgap between the tooth and the restoration to the length of the pulpal floor was calculated for each specimen (%SP) (Figure 4). The %SP was calculated as (silver nitrate penetration length/pulpal floor length) × 100.

Table 2: *Amount of Linear Shrinkage and Shrinkage Rate (SD), n=10**

Code	Average, μm	Strain Rate, μm/sec
P9	3.42 (0.91)[a]	0.43 (0.04)[a]
GD	13.40 (1.25)[b]	1.24 (0.15)[b]
Z3	10.98 (1.03)[b]	1.09 (0.19)[b]
CH	23.38 (1.39)[c]	1.87 (0.08)[c]
SD	25.40 (1.82)[c]	2.15 (0.14)[d]
TF	35.48 (3.42)[d]	2.88 (0.22)[e]

Abbreviations: P9, Filtek P90; GD, Gradia Direct; Z3,Filtek Z350; CH, Charisma; SD, SureFil SDR; TF, Tetric N- flow
** Different letters indicate different linear shrinkage values at p<0.05 level.*

Stereomicroscope Evaluation After Sectioning—To validate the results obtained from the micro-CT, teeth were prepared for stereomicroscope analysis. After the specimens were embedded in an acrylic resin, they were sectioned buccolingually using a low-speed diamond saw (500 rpm, Isomet, Buehler, IL, USA) and then polished with 1200-grit SiC paper. After embedding the samples in a 1% rhodamine solution for 24 hours, the resulting microgaps were evaluated using a stereomicroscope (Leica S8APO, Leica Microsystems, Wetzlar, Germany) at 120x magnification. The ratio of the rhodamine and silver nitrate penetration length into the microgap to the full pulpal floor length, was calculated for each specimen (%RP). The %RP was calculated as (rhodamine penetration + silver nitrate penetration length/pulpal floor length) × 100.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare the polymerization shrinkage strain and stress among the groups. Scheffe analysis was used for post hoc analysis. Additionally, polymerization shrinkage strain and stress rate was calculated at 10 seconds of light curing, and then they were compared using the same statistical method.

One-way ANOVA was used to compare the %SP among the groups before and after loading. A paired *t*-test was used to compare the %SP before and after thermomechanical loading. Scheffe analysis was used for post hoc analysis. All statistical inferences made were within a 95% confidence interval.

Pearson correlation analysis was used to compare the correlation between polymerization shrinkage strain/stress and %SP or %RP. The correlation between %SP after thermomechanical loading and %RP was also evaluated.

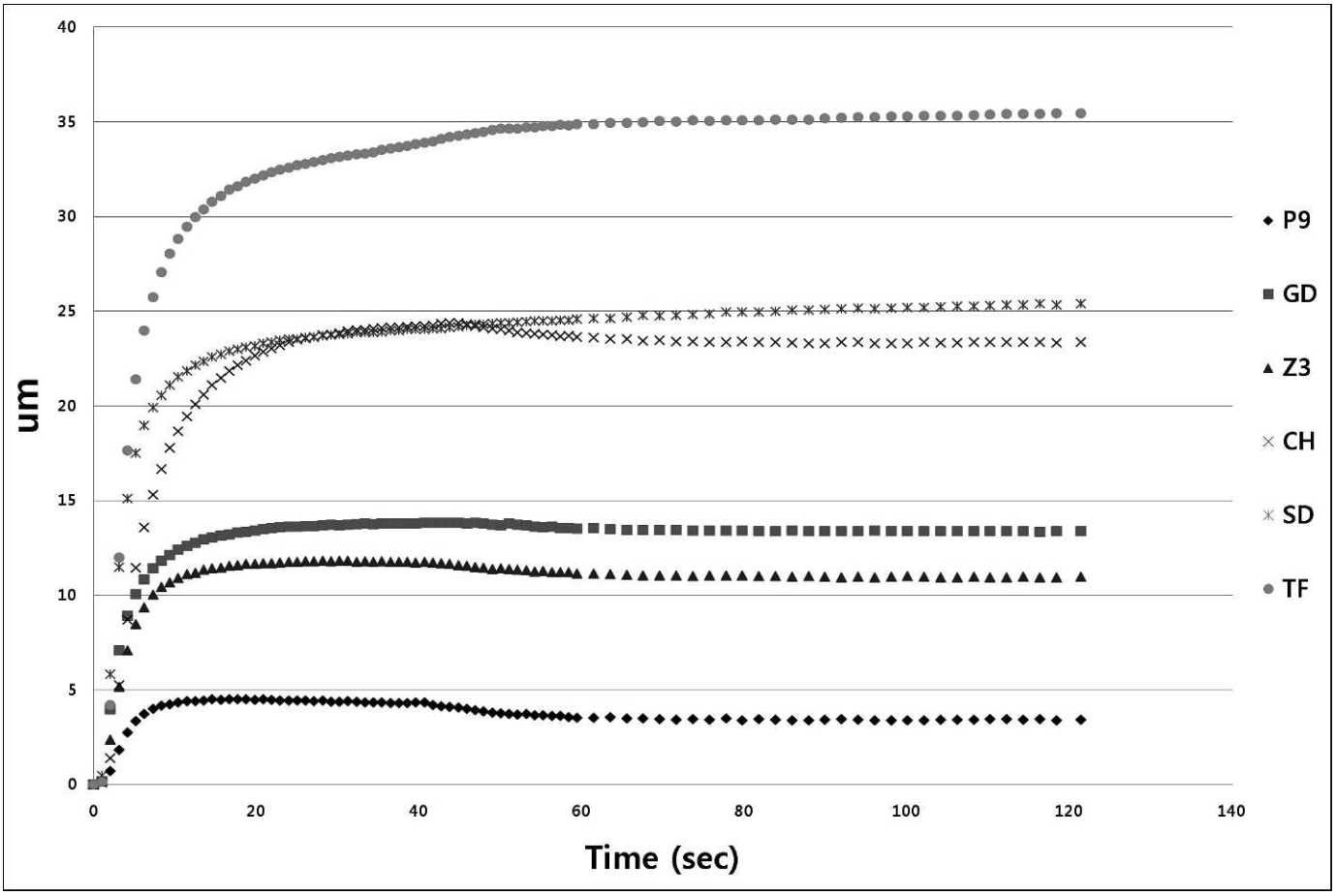

Figure 5. *The change of the linear polymerization shrinkage vs time.*

RESULTS

Polymerization Shrinkage Strain

The amount of polymerization shrinkage strain and strain rate are summarized in Table 2. The amount of polymerization shrinkage strain from least to greatest was P9 < Z3 ≤ GD < CH ≤ SD < TF ($p<0.05$). The shrinkage strain rate was P9 < Z3 ≤ GD < CH < SD < TF ($p<0.05$). The pattern of polymerization shrinkage strain for the materials is shown in Figure 5.

Table 3: *Amount of Shrinkage Stress and Stress Rate (SD), n=8**

Code	Stress, kgf	Stress rate, kgf
P9	1.78 (0.45)[a]	0.11 (0.04)[a]
GD	2.28 (0.24)[ab]	0.13 (0.02)[ab]
Z3	2.48 (0.15)[bc]	0.14 (0.02)[bc]
CH	2.87 (0.31)[bc]	0.15 (0.01)[bc]
SD	3.07 (0.15)[c]	0.17 (0.01)[c]
TF	4.01 (0.37)[d]	0.22 (0.02)[d]

Abbreviations: P9, Filtek P90; GD, Gradia Direct; Z3,Filtek Z350; CH, Charisma; SD, SureFil SDR; TF, Tetric N- flow
** Different letters indicate different shrinkage stress values at p<0.05 level.*

Polymerization Shrinkage Stress

The amount and rate of polymerization shrinkage stress are summarized in Table 3. The amount and rate of polymerization shrinkage stress from least to greatest was P9 < Z3 ≤ GD < CH ≤ SD < TF ($p<0.05$). The pattern of polymerization shrinkage stress for the studied materials is shown in Figure 6.

General Aspects of Silver Nitrate Penetration

In the nonflowable resin group (GD, P9, Z3, and CH), the silver nitrate solution was distributed uniformly throughout the entire pulpal floor region, and a few air bubbles were observed inside the composite resin. The amount of silver nitrate penetration increased after mechanical loading; this change in the penetra-

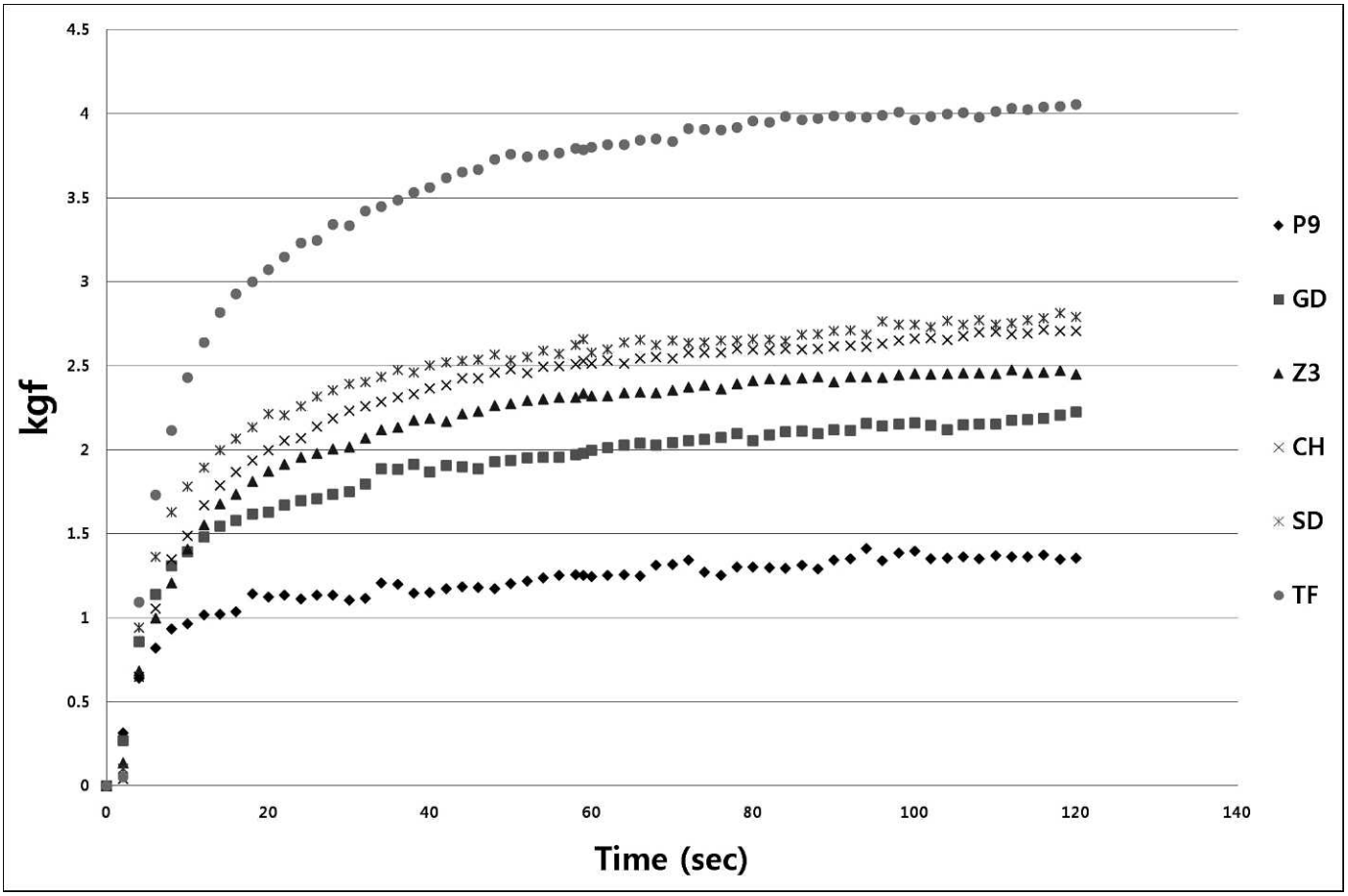

Figure 6. *The change of the polymerization stress vs time.*

tion quantities appeared to be the largest in the Z3 group.

In the flowable resin group (SD and TF), few air bubbles or defects were observed within resin composite material. The silver nitrate solution primarily penetrated the axio-pulpal line angle regions compared with the nonflowable resin. Before mechanical loading, a large amount of silver nitrate penetrated the specimens (Figure 7). However, the change in the penetration quantities after mechanical loading was smaller than in the nonflowable group.

%SP Evaluation

Table 4 and Figure 8 list the mean %SP and the associated standard deviations. The materials were ranked by %SP in the order P9 $\leq$ GD $\leq$ Z3 $<$ CH $\leq$ SD $<$ TF ($p<0.05$) before mechanical loading and P9 $\leq$ GD $\leq$ Z3 $\leq$ CH $\leq$ SD $<$ TF ($p<0.05$) after mechanical loading. In all groups, there was a significant difference between the values before and after mechanical loading ($p<0.05$).

%RP

The materials were ranked by %RP in the order P9 $\leq$ GD $\leq$ Z3 $\leq$ CH $\leq$ SD $\leq$ TF ($p<0.05$) (Table 5). The %RP had higher values and standard deviations than the %SP. The thickness of adhesive agent was between 60 and 90 μm in the middle area of the cavity floor and between 80 and 110 μm in the cavity corners.

Correlation Analysis

There was a positive correlation between the polymerization shrinkage strain/stress and %SP or %RP ($p<0.001$) (Table 6). They all showed medium to high correlations. There was a positive correlation between the %SP and %RP evaluation models ($p<0.001$, Pearson correlation constant 0.810).

DISCUSSION

The polymerization shrinkage of resin composite materials generates stress at the tooth-restoration

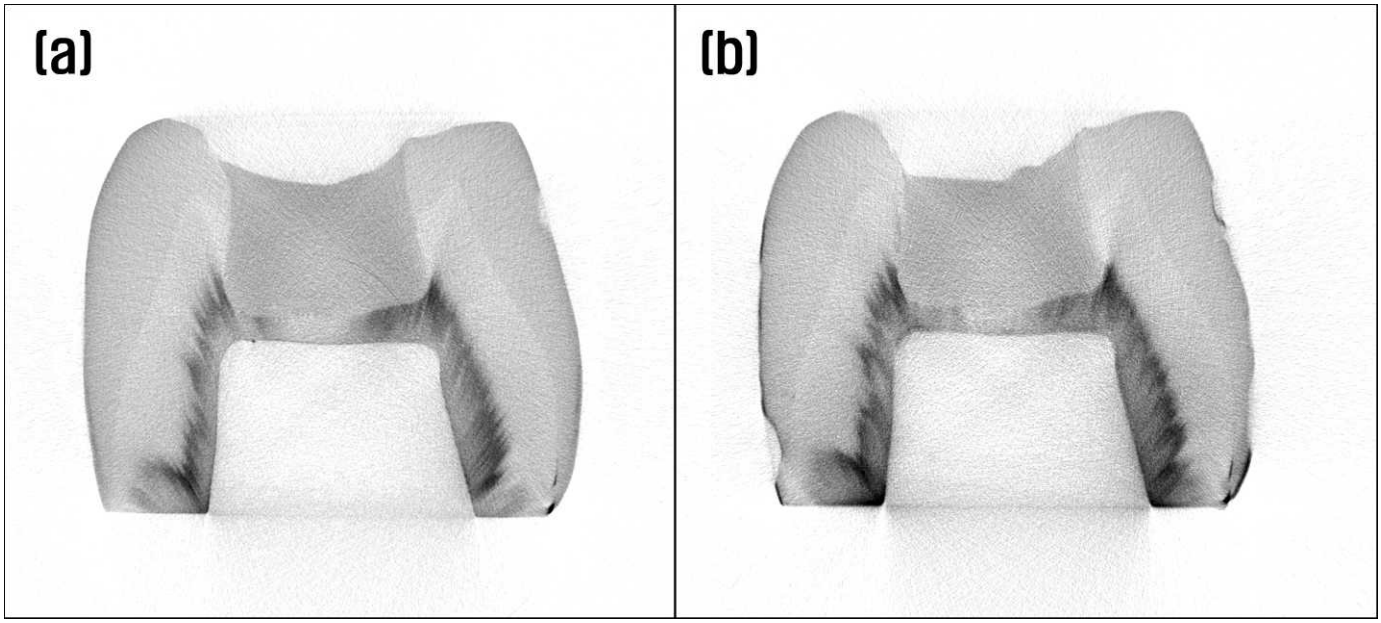

Figure 7. *Silver nitrate penetration. (a): Before mechanical loading. (b): After mechanical loading.*

interface and may clinically lead to the formation of marginal gaps. Several studies have been performed addressing the clinical relevance of this phenomenon with respect to *in vitro* microleakage. The correlation between polymerization shrinkage strain and microleakage[12-14] and the correlation between polymerization shrinkage stress and microleakage[15,16] have been evaluated. However, these studies have generally evaluated the microleakage of the marginal gap rather than the internal adaptation. To evaluate the correlation between the polymerization shrinkage strain/stress results and the internal adaptation generated by tooth stress in the present study, silver nitrate infiltration and micro-CT analysis were used. The factors influencing the stress formation included the volumetric polymerization shrinkage, the elastic modulus, the configuration factor of the restoration, the curing method used, and the adherence of the resin composite to the cavity walls.[17] In the present study, the cavity size and type, the curing mode, and the dentin adhesive were uniform across all specimens; only the resin composite material was varied.

Table 4: *Mean Percent and SD of Silver Nitrate Penetrate Length to Whole Pulpal Wall Length (%SP)†*

Thermomechanical Loading

Code	Before		After	
	Average	SD	Average	SD
P9	17.6[a]	2.4	24.4[a]	3.3*
GD	20.6[a]	2.8	26.8[a]	2.9*
Z3	21.9[a]	4.9	29.5[ab]	5.4*
CH	28.0[b]	4.4	34.7[bc]	3.4*
SD	33.8[b]	5.9	38.0[c]	6.3*
TF	46.0[c]	10.0	49.8[d]	10.1*

Abbreviations: P9, Filtek P90; GD, Gradia Direct; Z3,Filtek Z350; CH, Charisma; SD, SureFil SDR; TF, Tetric N- flow
† Eight teeth were used for each group and 100 images were used for each tooth for %SP analysis. Different letters indicate different penetration % level among filling materials ($p<0.05$) before and after thermomechanical loading, respectively.
** Significant differences in penetration %between before and after thermomechanical loading ($p<0.05$).*

The micro-CT data collection and the silver nitrate penetration length measurements were performed according to previous study protocols.[11] Because a restorative material with a radio-density similar to that of dentin might show background noise,[18] sagittal and coronal images of the same phase were compared with the DataViewer (Sky-Scan) program. The materials were ranked by %SP in the order P9 $\leq$ GD $\leq$ Z3 $<$ CH $\leq$ SD $<$ TF ($p<0.05$) before thermomechanical loading and P9 $\leq$ GD $\leq$ Z3 $\leq$ CH $\leq$ SD $<$ TF ($p<0.05$) after the loading. Before thermomechanical loading, %SP might show the internal adaptation of the initial condition. P9 had a lower %SP and TF had a higher %SP than the other groups. All of the nonflowable resins (P9, GD, and Z3) except CH had a lower %SP than the flowable resins (SD, TF) ($p<0.05$). After thermocycling loading, the ranks of the materials were similar to those before thermomechanical loading. It implies that the initial internal adapta-

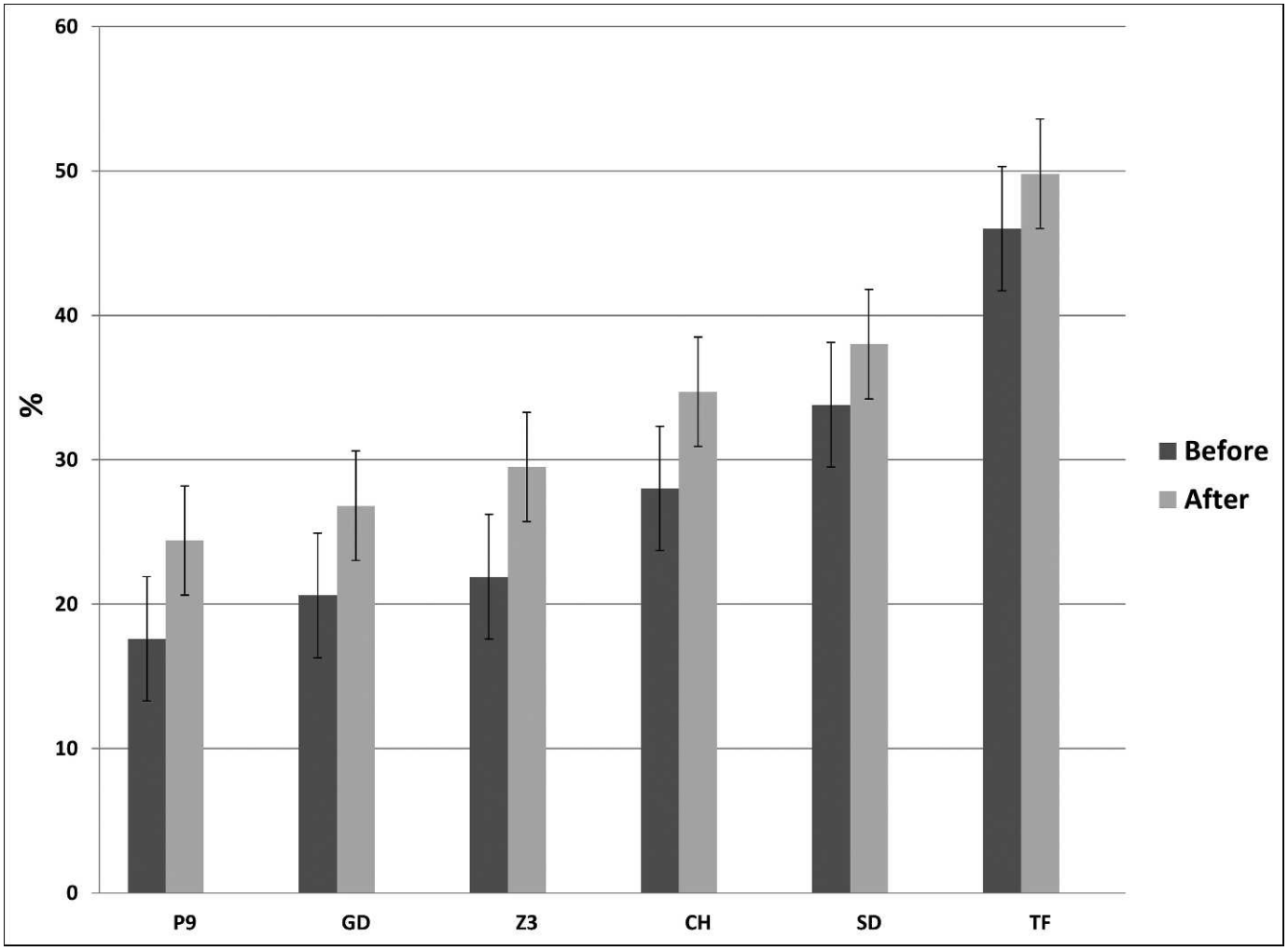

Figure 8. *Mean percent (%) and standard deviation of the silver nitrate penetration length relative to the pulpal wall length before and after thermomechanical loading.*

tion may affect the adaptation after thermomechanical loading. Considering the high correlation between %SP and polymerization shrinkage stress and strain (Table 6), the higher %SP values in flowable composites seems to be related with higher shrinkage strain/strain values than nonflowable composites.

Table 5: *Mean Percent and SD of Total Rhodamine Penetrate Length to Whole Pulpal Wall Length (%RP)) (n=8 for Each Group)**

Code	Total Microgap	SD
P9	34.4[a]	10.8
GD	36.7[a]	9.2
Z3	37.1[a]	9.1
CH	41.7[ab]	11.8
SD	49.1[ab]	11.9
TF	55.3[ab]	10.9

Abbreviations: P9, Filtek P90; GD, Gradia Direct; Z3,Filtek Z350; CH, Charisma; SD, SureFil SDR; TF, Tetric N- flow
** Different letters indicate different microgap values at $p<0.05$ level.*

In the present study, a chewing simulator was used to simulate clinical situations. In all groups, there was a significant difference before and after thermomechanical loading, and it was consistent with the results of a previous study.[11] The difference in %SP before and after mechanical loading was 3%-4% in the flowable resin groups (SD and TF) and 6%-8% in the nonflowable composite groups (GD, P9, Z3, and CH). This difference might be due to the stress-absorbing ability of the flowable resin, which has a low elastic modulus, making it possible to minimize the destruction of the adhesion of the restoration.[19] After thermomechanical loading, the correlation between the polymerization shrinkage strain and stress and the %SP was reduced (Table 6). Mechan-

Table 6: *Pearson Correlation Constant*[a]

Thermomechanical Loading	%SP		%RP
	Before	After	After
Polymerization shrinkage strain	0.805	0.777	0.655
Polymerization shrinkage stress	0.819	0.785	0.686

Abbreviations: P9, Filtek P90; GD, Gradia Direct; Z3,Filtek Z350; CH, Charisma; SD, SureFil SDR; TF, Tetric N- flow
[a] *%SP is equal to (silver nitrate penetration length/pulpal floor length)×100. %RP is calculated as (rhodamine penetration + silver nitrate penetration length/pulpal floor length)×100.*

ical properties such as the elastic modulus might affect the results in this period.

In the flowable resin groups, silver nitrate penetration was concentrated in the axio-pulpal line angle regions. These results are similar to those obtained from a photoelastic model that measured the stress distribution of teeth with resin composite restorations.[20] Another possible cause might be a difference in the application method. Whereas hand instruments were used to pack the composites into the cavities in the nonflowable composites, the flowable resins were injected into them, and packing was therefore not necessary.[21]

To validate the results obtained from the micro-CT, all specimens were sectioned and evaluated by stereomicroscope. There was a high correlation between the %RP and the %SP ($p<0.001$, Pearson correlation constant 0.810). This observation shows that the micro-CT and silver nitrate penetration techniques can be used alternatively for the evaluation of internal adaptation. In certain cases in which silver nitrate had previously penetrated the gap, it was very difficult to detect the rhodamine penetration because the intense black shade of the silver nitrate could prevent the detection of the red rhodamine shade. In these cases, the silver nitrate penetration length was added to the rhodamine penetration length for the %RP calculations because the microgap was evident.

In all cases, the %RP had a higher value than the %SP. This result might be due to the destructive nature of the sectioning process. This may also show the limitations of the dye penetration and sectioning methods. The correlation between the %RP and the polymerization shrinkage strain and stress was lower than that of the %SP (Table 6). It is assumed that the destructive sectioning procedure affected the %RP.

The factors that influence polymerization shrinkage include monomer molecular weight and concentration and filler size and concentration.[22] GD, P9, Z3, and CH all have higher filler content than the flowable composites, SD and TF. These high-filler resin composites have a lower monomer content that participates in the polymerization process, which is related to the lower polymerization shrinkage. Although the space occupied by the filler particles does not participate in the curing contraction, high filler loads may require low-molecular-weight monomers to ensure a proper handling viscosity. In low-viscosity materials, the motility of the monomers is active, such that a higher proportion of monomers participates in the polymerization process, increasing the polymerization shrinkage.[23] In the present study, CH exhibits significantly higher levels of polymerization shrinkage strain than the other nonflowable resins tested because of its high TEGDMA level.[24] The addition of low molecular-weight TEGDMA to decrease the viscosity contributes to a higher level of polymerization shrinkage than GD and Z3, which contain high molecular-weight monomers such as UDMA and Bis-EMA.

Many efforts to minimize polymerization stress by changing the resin composite formulation have been reported.[25-28] These efforts include the introduction of high-molecular-weight monomers (TCD-urethane or dimer dicarbamate dimethacrylate),[25] silorane-based resin composite,[26] and SDR (stress decreasing resin).[27,28] P9 is a silorane-based resin composite composed of a siloxane core and an oxirane ring. Its polymerization occurs via a cationic ring-opening reaction, resulting in a lower polymerization contraction compared with those of methacrylate-based resins, which polymerize via a radical addition reaction of their double bonds. The cationic cure starts with the generation of an acidic cation that opens the oxirane ring and generates a new acidic carbocation center. After the addition to an oxirane monomer, the epoxy ring is opened to form a chain or, in the case of two or multifunctional monomers, a network.[26] Volumetric expansion occurs during this process and compensates for the polymerization shrinkage. Because of the specific structure of P9, the manufacturer recommends using the P9 system adhesive self-etch primer.[29]

In this study, the XP bond was applied for evaluation in the same conditions as the other resin composites. It is not clear why there was relatively good internal adaptation, even though XP bond was used in P9. It might be connected with relatively lower polymerization shrinkage in P9 and poorer chemical reaction between XP bond and P9. Limited amounts of shrinkage stress would be experienced at the adhesive and the composite interface and this

would lead to better sealing by the underlying adhesive. Further study will be necessary.

Here, P9 has a lower polymerization shrinkage strain than the other five groups, and similar results have been previously reported.[30] In terms of the polymerization shrinkage stress, it has been reported that P9 does not show the lowest shrinkage stress.[31,32] It has also been reported that low-shrinkage resin composite materials may have less volumetric shrinkage but that their polymerization shrinkage stress is still similar to that of conventional resin composites.[33] This difference might be explained by the fact that the polymerization shrinkage stress was affected not only by volumetric shrinkage but also by the elastic modulus of the material, gel times, and so on. Resin composites that have the same volumetric shrinkage do not always generate the same stress. A resin composite with a higher elastic modulus generates higher polymerization shrinkage stress because the flow in the resin material is limited.[26] In the present study, however, P9 also showed the lowest polymerization shrinkage stress. This result might be explained by the low polymerization shrinkage strain compared with those of the methacrylate-based resin composites and the property, which delays the gel point.[34] P9 took the longest time to reach the gel point because of the time needed for cation formation; siloranes have a polymerization reaction with a slow onset. This means that the silorane resin composite possessed the highest potential for stress relief by permitting material flow during the initial curing stage.[34] As a result, P9 exhibited the lowest shrinkage stress, although P9 also showed a high elastic modulus of 9 GPa.

Comparing the results of polymerization shrinkage strain and stress, there was similarity in terms of material ranking. However, the order of Z3 and GD was changed. This discrepancy may be explained by differences in the elastic modulus of the resin composite and the polymerization shrinkage strain. For example, in the present study, the elastic modulus of Z3 was 11 GPa (Table 1) and its linear shrinkage was 10.4 μm, while the elastic modulus of GD was 6.3 GPa and its linear shrinkage was 13.40 μm. When the elastic modulus × linear shrinkage was calculated, which affects the polymerization shrinkage stress, this value was 114.4 GPa in Z3 and 84.42 GPa in GD. In the nonflowable resins Z3, GD, and CH, the composition of the resin composite affected its elastic modulus. The elastic moduli of the dimethacrylate polymers can be ranked as follows: TEGDMA < Bis-EMA < UDMA < Bis-GMA.[35] The UDMA-based resin composite GD exhibits lower polymerization shrinkage stress than the Bis-GMA–based resin composite Z3. It is not surprising that resin composites with higher polymerization shrinkage strain had higher polymerization shrinkage stress. However, the polymerization shrinkage stress is also affected by the composition of the resin composite and the elastic modulus, giving different results, according to previous findings.[36]

SD was reported to lower the polymerization rate and fill up to a 4-mm bulk filling.[27] Study on SD has shown that it has reduced volumetric shrinkage compared with conventional methacrylate-based flowable composites,[28] and it is consistent with the results of the present study. SD is composed of a urethane dimethacrylate structure that is responsible for the reduction in polymerization shrinkage and stress. This effect may be due in part to the larger size of the SD resin compared with TF (molecular weight of 849 g/mol for SD resin compared with 513 g/mol for Bis-GMA). The properties of SD are due to the combination of the large molecular structure with a chemical moiety called a "polymerization modulator" that is chemically embedded in the center of the polymerizable resin backbone of the SD monomer. The high molecular weight and the conformational flexibility around a centered modulator impart optimized flexibility, such that SD shows a lower polymerization shrinkage than methacrylate flowable resin.[37] In the limited results of the present study, SD showed superior internal adaptation to that of the conventional flowable resin TF, but it showed inferior internal adaptation compared with other nonflowable composites except CH. Further study will be necessary for clinical determination of its applicability.

In this study, the strain and stress rates were calculated at 10 seconds of light curing. The statistical results of polymerization shrinkage strain and stress were very similar to those of strain and stress rate (Tables 2 and 3). It means that early stress and strain rate are important factors to determine final shrinkage stress and strain.

The four null hypotheses in this study could be rejected because there were positive correlations between the %SP, the %RP, and polymerization shrinkage strain and stress (Table 6, $p<0.001$).

The proposed method, in which a silver nitrate solution was used to penetrate the pulp space through the dentinal tubules and the resulting amount of penetration into the microgap areas was assessed by micro-CT, may provide a new measure

for evaluating internal adaptation nondestructively. In addition, this method has the advantage that constant results were obtained reproducibly both before and after thermomechanical loading.

CONCLUSION

In this study, the polymerization shrinkage stress and strain were found to be closely related to the internal adaptation of the resin composite restorations. The newly proposed model for the evaluation of internal adaptation using micro-CT and silver nitrate may provide a new measurement for evaluating the internal adaptation of restorations in a nondestructive way.

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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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Incremental Filling Technique and Composite Material—Part I: Cuspal Deformation, Bond Strength, and Physical Properties

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Clinical Relevance

The use of a large number of increments caused an increase in cuspal deflection during composite polymerization in large posterior restorations. A balance between adequate bonding with good mechanical properties of the composite and lower cuspal deformation was obtained with 2.0-mm increments.

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SUMMARY

Objectives: To evaluate the effect of composite resins (one conventional and two low-shrink composites) and filling techniques on cuspal strains (CS), microtensile bond strength (μTBS), composite ultimate tensile strength (UTS), and mechanical properties of the composites at various depths in molars with large Class II restorations.

Materials and Methods: One hundred seventeen human molars received standardized Class II mesio-oclusal-distal cavity preparations and restorations with three composites (Filtek LS [3M-ESPE]; Aelite LS [BISCO]; and Filtek Supreme [3M-ESPE]) using three filling techniques (bulk, eight increments, and 16 increments). CS was measured using strain gauges, after which the same restored teeth were used to assess μTBS and UTS. The elastic modulus (E) and Vickers hardness (VH) at different depths were determined from microhardness indentations. The CS, μTBS, UTS, E,

and VH data were statistically analyzed using split-plot analysis of variance and Tukey test (p=0.05).

Results: The CS was higher when using 16 increments. The 'low-shrink' composites caused lower CS. The μTBS and UTS were similar for eight- and 16-increment techniques and higher when compared to the bulk filling in all composites. E and VH were constant through the depth when applied in eight or 16 increments.

Conclusions: Type of composite and filling technique affected the CS, μTBS, UTS, and mechanical properties of large Class II restorations. The eight-increments filling technique resulted in generally less CS with the same μTBS and UTS than was obtained with 16 increments, without affecting E and VH through the depth of the composites.

INTRODUCTION

Volumetric shrinkage is a consequence of the polymerization process, whereby the conversion of monomer molecules results in a cross-linked polymer network.[1] During this polymerization reaction, the composite changes from a predominantly viscous to a predominantly solid substance, which can be characterized by the development of the elastic modulus (E).[2] Residual shrinkage stresses can evolve when volumetric polymerization contraction is accompanied by this E development and the surrounding tooth structure restricts the volumetric changes.[3,4] It is generally believed that these prestressed restorations will have adverse clinical consequences.[5] Polymerization shrinkage of composite resins has been a clinical concern, and the associated stresses are thought to play a role in marginal failures, microleakage, and recurrent caries.[4-8] On the other hand, other studies[9,10] found a low degree of correlation between clinical failures and composite shrinkage. Marginal gaps created by polymerization shrinkage did not appear to increase the risk for secondary caries but can lead to marginal staining, which may be diagnosed as secondary caries.[9]

Shrinkage stresses manifest themselves most directly in cuspal deflection of restored teeth.[1,11] Cuspal deflection usually increases with increasing cavity dimensions and signifies an increased risk of tooth fracture.[12-14] How much stress is generated by polymerization shrinkage depends on multiple factors, such as curing light intensity, photoactivation time, mechanical properties of materials and tooth structure, and restorative placement technique, as well as the geometry and extent of the cavity.[2,15]

New low-shrink composite resins and restorative protocols have been developed to minimize polymerization shrinkage and stress. Incremental filling techniques have often been indicated to decrease the effects of shrinkage and stress generated at the adhesive interface.[16] However, a study[2] using finite element analysis showed that incrementally filling could produce higher shrinkage stresses at the adhesive interface. On the other hand, light-curing large restorations in bulk raises concerns with regard to whether the composite can be adequately cured throughout the whole restoration depth because increasing the depth of an increment or restoration decreases the light intensity that reaches the bottom of such layers and consequently may not reach an adequate degree of conversion.[17] An inadequate cure compromises the mechanical properties of a composite restoration and its adhesion and, consequently, its long-term clinical success.[18]

Questions also remain about how shrinkage stresses may compromise the interface and/or the mechanical properties of a restoration. Both the adhesion to tooth substrate and the mechanical properties of a restoration might reflect the contraction stress behavior of a composite resin during polymerization.[19,20]

Assessment of the effect of composite resins and bulk/incremental filling techniques on the mechanical performance of restored teeth therefore requires a systematic and comprehensive study of the tooth deformation, bond strength, and mechanical properties. The purpose of this study was to investigate those factors in molars with an extensive Class II restoration. The null hypothesis was that the restorative materials and filling techniques would not affect cuspal deformation, bond strength, and mechanical properties in restored molars.

MATERIALS AND METHODS

Teeth Selection and Cavity Preparation

One hundred seventeen extracted intact, caries-free human third molars were used with approval from the University Ethics Committee in Human Research. The teeth were selected to have an intercuspal width that fell within a maximum deviation of not more than 10% of the determined mean. The measured intercuspal width varied between 5.17 mm and 6.13 mm. The teeth were embedded in a polystyrene resin (Cristal, Piracicaba, SP, Brazil) up to 2.0 mm below the cervical line to simulate alveolar

Table 1: *Dental Composites Tested in the Study (Information Provided by the Respective Manufacturers)*

Composite Resins	Wt%	Vol%	Filler Type	Matrix	Manufacturer
Filtek LS (LS)	76	55	Quartz and yttrium fluoride (0.1-2.0 μm)	TEGDMA, ECHCPMS	3M ESPE, St Paul, MN, USA
Aelite LS Posterior (AE)	84	74	1.1 μm	Bis-GMA, UDMA	BISCO, Schaumburg, IL, USA
Filtek Supreme (SU)	82	60	Silica nanofillers (75 nm) zirconia nanofillers (5-10 nm) and agglomerated zirconia/silica nanoclusters (600-1400 nm)	Bis-GMA, Bis-EMA, UDMA, TEGDMA	3M ESPE, St Paul, MN, USA

Abbreviations: Bis-EMA, bisphenol-A hexaethoxylated dimethacrylate; Bis-GMA, bisphenol-A glycol dimethacrylate; ECHCPMS, 3,4-epoxycyclohexylcyclopolymethylsiloxane; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

bone.[21] The teeth were cleaned using a rubber cup and fine pumice water slurry and distributed into nine groups of 13 teeth apiece. Ten teeth per group were restored and used for cuspal deflection measurement using strain gauges and afterwards for the bond strength and ultimate tensile strength of the composite restoration using a microtensile test. The other three teeth per group were restored and used for the measurement of Vickers hardness (VH) and E using the continuing indentation method. All restored teeth had Class II cavities with 4.5-mm intercuspal width and 5-mm depth, prepared with a diamond bur (#3099 diamond bur, KG Sorensen, Barueri, SP, Brazil) in a high-speed handpiece with copious air-water spray using a cavity preparation machine.[22] This machine consisted of a high-speed handpiece (EXTRA torque 605 C; KaVo do Brasil, Joinvile, SC, Brazil) coupled to a mobile base. The mobile base moves vertically and horizontally with three precision micrometric heads (152-389; Mitutoyo Sul Americana Ltda, Suzano, Brazil), attaining a 0.002-mm level of accuracy.

Cuspal Strain (CS)

Cuspal deformation was measured with strain gauges (PA-06-060CC-350L, Excel Sensores, SP, Brazil), which had an internal electrical resistance of 350 Ω, a gauge factor of 2.07, and a grid size of 21.02 mm^2. The gauge factor is a proportional constant between electrical resistance variation and strain. The strain gauges were bonded to the cervical area of the buccal and lingual surfaces (n=10) with cyanoacrylate adhesive (Super Bonder; Loctite, São Paulo, Brazil), and the wires were connected to a data acquisition device (ADS0500IP; Lynx Tecnologia Eletrônica, São Paulo, SP, Brazil). The strain gauges were placed in the region where a finite element model had indicated the presence of the highest polymerization stresses.[23] In addition, two strain gauges were fixed to another intact tooth to compensate for dimensional deviations due to temperature effects.

The materials used in this study were two low-shrink composite resins, Filtek LS (LS) and Aelite LS (AE), and one conventional composite resin, Filtek Supreme (SU). Their composition and manufacturer information are listed in Table 1. Adhesive systems specific to each composite (LS: S System Adhesive Self-Etch Primer and Bond [3M ESPE, St Paul, MN, USA]; AE: All-Bond SE [Bisco, Schaumburg, IL, USA]; and SU: Adper Easy one [3M ESPE]) were used according to the manufacturers' instructions. The cavities were restored using three filling techniques: bulk, eight increments, and 16 increments. Average volumes of composite per increment for each technique were 221.3 mm^3, 27.66 mm^3, and 13.83 mm^3, respectively. A Teflon matrix with the cavities was made to standardize each composite resin increment before the insertion into the cavity (Figure 1). Each increment was light-cured for 40 seconds using a light source with 550 W/cm^2 output (Demetron Kerr; Orange, CA, USA) by placing from the occlusal direction closest to the cavity. The total energy for each filling technique was 22 J/cm^2 for the bulk technique; 176 J/cm^2 for the eight-increment technique, and 352 J/cm^2 for the 16-increment technique. The cuspal deformation data were obtained from the strain gauges through data analysis software (AqDados 7.02 and AqAnalisys; Lynx). The strain values were recorded at 4 Hz during the restorative procedure and continued for 10 minutes after curing the last increment.

Bond Strength (μTBS) and Ultimate Tensile Strength (UTS)

The restored teeth were stored for 24 hours in distilled water at 37°C, after which the occlusal surface was removed and discarded. The specimens were sectioned bucco-lingually into six slabs of 1 mm in thickness using a low-speed diamond saw (Isomet,

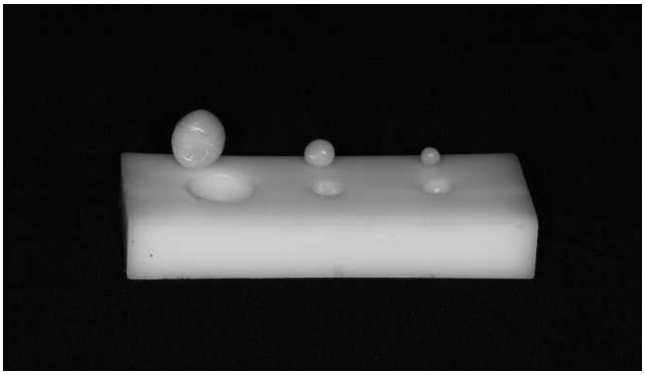

Figure 1. *A Teflon matrix made to standardize each composite resin increment before the insertion into the cavity.*

Buehler, Lake Bluff, IL, USA) under water cooling. Each slab was serially sectioned horizontally to harvest two sticks with 1.0 mm × 1.0 mm cross sections at two cavity depths (six sticks for each depth). A top stick represented the upper 2 mm occlusal region of the dentin/composite interface, and a bottom stick represented the bottom 2 mm in the cervical region of the dentin/composite interface. In each experimental group, half of the sticks from each depth were subjected to the μTBS test (n=30; 10 teeth per group, three sticks per region). The other half of the sticks were trimmed to an hourglass shape at the center of the restorative material and used for the measurement of the UTS of the composite resin (Figure 2).

For the μTBS and UTS tests, the ends of the specimen were glued to a microtensile device in the testing machine (EMIC DL 2000, São José dos Pinhais, Paraná, Brazil) using cyanoacrylate glue (Super Bonder Flex Gel, Henkel Loctite Adesivos Ltda, Itapevi, SP, Brazil) to cover all the faces of the specimens[24,25] and were then subjected to a tensile load at a crosshead speed of 1 mm/min. The cross-sectional area of each stick was measured using a digital caliper (Mitutoyo CD15, Mitutoyo Co, Kawasaki, Japan). The μTBS and UTS were calculated by dividing the fracture load by the surface area, measured to the nearest 0.01 mm with the digital caliper.

After the μTBS test, the specimens were examined with a stereomicroscope (Leika Ecafix, Tokyo, Japan) at 40× magnification. The fractured surfaces were classified as cohesive failure in composite, adhesive failure, or mixed failure.

E and VH

The other three restored teeth from each group were used for the analysis of mechanical properties (E and VH) of the composites at five depths. Each restored tooth was sectioned in the buccal-lingual direction into two halves using a precision saw (Isomet, Buehler). One section per tooth was randomly selected for assessment of the mechanical properties. The specimens were embedded with methacrylate resin (Instrumental Instrumentos de Medição Ltda, São Paulo, SP, Brazil). Prior to testing, the surfaces were finished with silicon-carbide paper (#600, 800, 1200, and 2000 grit sizes; Norton, Campinas, SP, Brazil) and polished with metallographic diamond pastes (6-, 3-, 1-, and 1/4-μm sizes; Arotec, São Paulo, SP, Brazil). Using a Vickers indenter (CSM Micro-Hardness Tester; CSM Instruments, Peseux, Switzerland), indentations were made every 1.0 mm from 0.5 mm to 4.5 mm, measured from the pulpal wall of the restorations. The indentation was carried out with controlled force, whereby the test load was increased or decreased at a constant speed ranging between 0 and 500 mN in 20-second intervals. The maximum force of 500 mN was held for five seconds. The load and the penetration depth of the indenter were continuously measured during the load-unload-hysteresis.

The universal hardness is defined as the applied force divided by the apparent area of the indentation at the maximum force. The measurements were expressed in VH units by applying the conversion factor supplied by the manufacturer. The indentation modulus was calculated from the slope of the

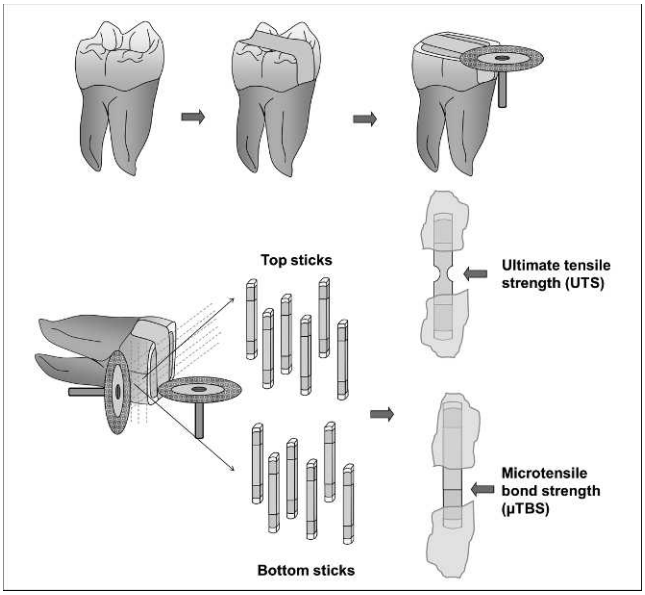

Figure 2. *Schematic illustration describing the sample preparation for the microtensile tests used to measure the microtensile bond strength and ultimate tensile strength at the top and the bottom of the cavities.*

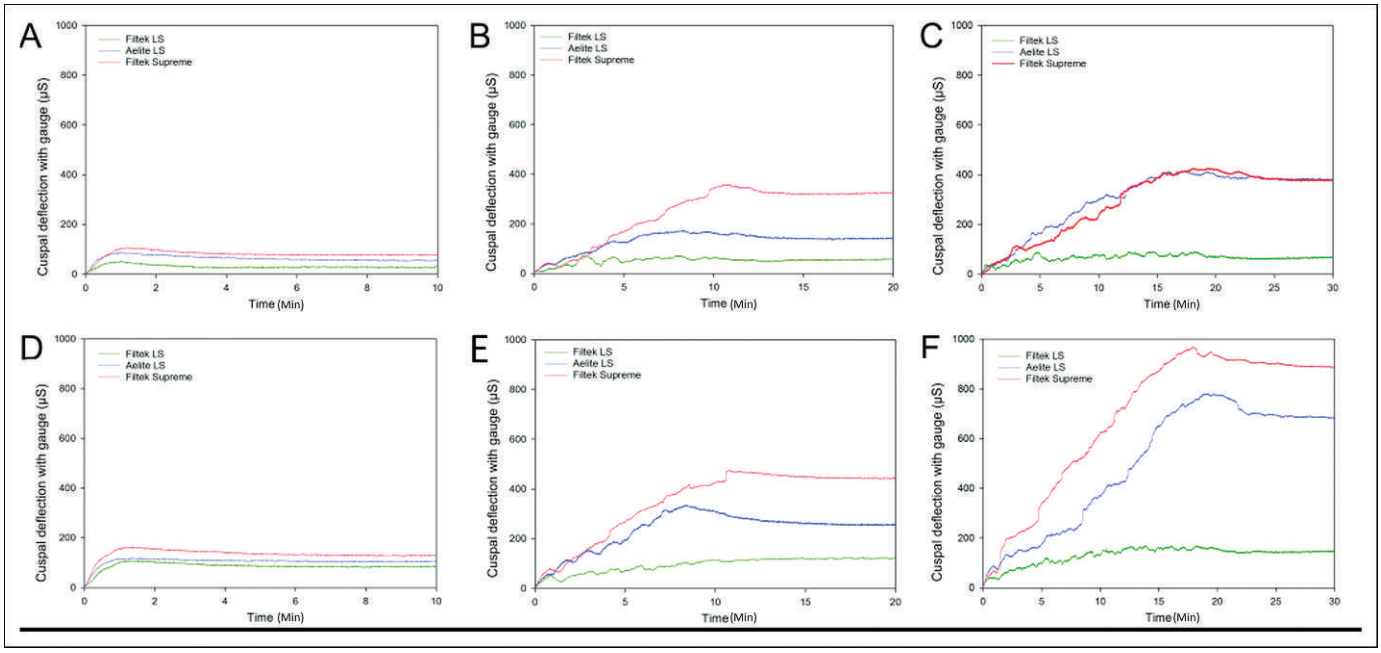

Figure 3. *Cuspal deformation in microstrains (µS) measured with strain gauges placed on the buccal or lingual cuspal surfaces. (A) Buccal cusp of the tooth filled in bulk; (B) buccal cusp of the tooth filled in eight increments; (C) buccal cusp of the tooth filled in 16 increments; (D) lingual cusp of the tooth filled in bulk; (E) lingual cusp of the tooth filled in eight increments; and (F) lingual cusp of the tooth filled in 16 increments.*

tangent of the indentation depth curve at the maximum force and is comparable to the E of the material.[26]

Statistical Analysis

The cuspal deflection, µTBS, UTS, E, and VH data were tested for normal distribution (Shapiro-Wilk, $p>0.05$) and equality of variances (Levene test, $p>0.05$), followed by parametric statistical tests. Analysis of variance (ANOVA) was performed in a split-plot arrangement, with the plot represented by the composite resin, restorative technique, and their interaction and the subplot represented by depth of the cavity. Multiple comparisons were made using the Tukey test. The data of fracture mode were subjected to the chi-square test ($p<0.05$). All tests employed a 0.05 level of statistical significance, and all statistical analyses were carried out with the statistical package SAS® System version 9.1 (SAS Institute Inc, Cary, NC, USA).

RESULTS

CS

The behavior and values of the cuspal deformation (strain) for the three composites (LS, AE, and SU) and the three filling techniques (bulk and 8- and 16-increment) are shown in Figure 3 and Table 2. LS had the lowest values of cuspal deformation, followed by AE and SU in eight- and 16-increment filling techniques. This behavior was consistent for both buccal and lingual cusps, with the higher strain values for the lingual cusp for AE and SU. Cuspal deformation for the LS restored teeth was not significantly different among the three filling techniques. AE and SU had lower lingual cuspal

Table 2: *Cuspal Deformation (µS) Measured by Strain Gauges (n=10 Teeth)*[a]

Composite Resins	Mean (Standard Deviation)					
	Buccal			Lingual		
	Bulk	Eight Increments	16 Increments	Bulk	Eight Increments	16 Increments
Filtek LS	69.2 (34.4) Aa	86.3 (43.7) Aa	79.1 (49.9) Aa	124.8 (51.9) Aa	134.5 (41.7) Aa	167.5 (67.9) Aa
Aelite LS	106.3 (32.7) Aa	187.7 (73.4) Ab	406.4 (315.8) Bb*	140.5 (32.3) Aa	328.0 (169.4) Bb	652.2 (398.1) Cb*
Filtek Supreme	119.3 (49.8) Aa	373.5 (166.6) Bb	424.6 (246.5) Bb*	175.5 (60.9) Aa	509.6 (226.1) Bb	940.9 (761.2) Cc*

[a] *Different uppercase letters in rows are designed to compare restorative technique for each cusp; lowercase letters in columns are designed to compare composite resin for each cusp; * Significant difference for pairwise comparison between buccal and lingual cusps (p<0.05).*

Table 3: *Microtensile Bond Strength Mean Values (MPa) for Each Group (n=10 Teeth)*[a]

Composite Resins	Mean (Standard Deviation) Microtensile Bond Strength, MPa					
	Top of Restoration			Bottom of Restoration		
	Bulk	Eight Increments	16 Increments	Bulk	Eight Increments	16 Increments
Filtek LS	7.9 (3.1) Ca	17.3 (5.5) Bb	28.2 (6.8) Aa	5.6 (1.7) Cb	20.3 (6.1) Ba	27.8 (7.8) Aa
Aelite LS	8.7 (2.4) Ba	16.1 (5.0) Ab*	18.7 (7.0) Ab	6.7 (2.6) Bb	22.3 (6.6) Aa*	19.3 (8.3) Ab
Filtek Supreme	11.0 (5.7) Ba	29.2 (6.4) Aa*	24.4 (6.9) Aab	14.5 (5.2) Ba	22.3 (10.5) Aa*	25.9 (8.6) Aa

[a] *Different uppercase letters in rows are designed to compare restorative technique for each region, lowercase letters in columns are designed to compare composite resin for each region indicate significant differences; * Significant difference for pairwise comparison between top and bottom for each group (*$p<0.05$*).*

deformation when the bulk filling technique was used, followed by the eight- and 16-increment filling techniques.

μTBS

The μTBS values in MPa (mean and standard deviation) for the three composite resins, the filling techniques, and the regions of the cavity for the experimental groups are presented in Table 3. ANOVA revealed a statistically significant difference among the composite resins ($p<0.0001$), the filling techniques ($p<0.0001$), the interactions between composite resins and filling technique ($p=0.0064$), and the interactions between composite resin, filling technique, and region of cavity ($p=0.0094$).

LS had significantly higher μTBS with the 16-increment technique than with the other two techniques, and it had significantly lower μTBS with the bulk technique, regardless of top or bottom region. For AE and SU there was no significant difference between the eight- and 16-increment techniques, whereas the bulk technique had the lowest μTBS values, regardless of the cavity region.

No significant difference in μTBS among the three composite resins filled in bulk was found for the top of the restoration. However, SU had significant higher μTBS values than did the other composite resins at the bottom of the restorations. For the eight-increment technique, SU had significantly higher μTBS than for both low-shrink composite resins LS and AE at the top of the restoration; however, none of these values was significantly different at the bottom. For the 16-increment technique, LS had significantly higher μTBS than AE at the top of the restoration, and AE had significantly lower μTBS than both SU and LS at the bottom.

Fracture mode distributions and statistical differences are shown in Figure 4. All composites filled

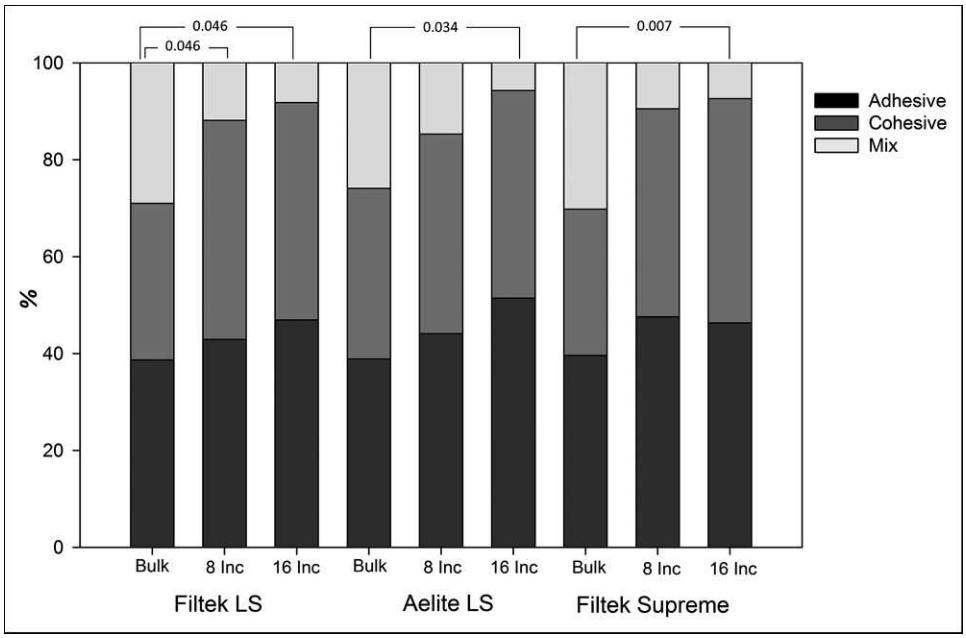

Figure 4. *Fracture mode distribution. T indicates the top region of the restoration; B is the bottom region of the restoration.* p *Values for significant differences found by chi-square test (*$p<0.05$*).*

Table 4: *Ultimate Tensile Strength (MPa) of the Composite Restoration (n=10)*[a]

Composite Resins	Mean (Standard Deviation) Ultimate Tensile Strength, MPa						Pooled Average
	Bulk		Eight Increments		16 Increments		
	Top	Bottom	Top	Bottom	Top	Bottom	
Filtek LS	17.4 (4.7)	13.8 (5.2)	42.1 (12.9)	41.9 (12.2)	40.1 (8.6)	36.7 (11.2)	33.3 (14.9) A
Aelite LS	16.9 (6.4)	17.8 (6.3)	38.4 (9.8)	39.6 (9.7)	40.7 (13.9)	42.6 (11.5)	32.0 (13.9) A
Filtek Supreme	13.1 (4.7)	12.1 (5.4)	43.6 (12.4)	37.5 (7.5)	45.5 (12.9)	44.3 (11.3)	32.4 (17.4) A
Pooled average	15.2 (5.7) B		40.5 (10.6) A		41.2 (11.2) A		

[a] *For the pooled averages, means followed by distinct letters are significantly different (Tukey test, 95% confidence level). None of the pairwise comparisons between the top and bottom values were significantly different.*

showed similar failure patterns, regardless of the filling technique used. The incidence of mixed failures was higher with the bulk technique compared to the 16-increment filling technique for all composites and compared to the eight-increment filling technique for FS.

UTS

The UTS values in MPa (mean and standard deviation) for the three composite resins, the filling techniques, and the regions of the cavity are shown in Table 4. ANOVA revealed a statistically significant difference only for the filling technique ($p<0.05$). The UTS was significantly lower with the bulk filling technique than with the eight- and 16-increment techniques, regardless of the composite resin and region of the cavity.

E

The E values in GPa for the three composites and the filling technique at various depths of the restorations are shown in Figure 5. The E of AE decreased in the bulk filling technique when the depth of the restoration increased, while the E of LS and SU was constant between the depths of 0.5 and 2.5 mm and was significantly decreased beyond 2.5 mm. For the eight-increment technique, LS and SU maintained a constant E throughout the depth of the restoration, while AE maintained the constant E up to 3.5 mm deep and decreased significantly for the 4.5-mm depth. For the 16-increment technique, the E was constant for all of the depths in all composites.

AE had significantly higher E values than did SU and LS up to 1.5 mm in depth for the bulk filling technique and had significantly higher E values at all depths with the eight- and 16-increment techniques. SU and LS showed similar E values for the bulk filling technique, regardless of the restoration depth. However, for the eight- and 16-increment techniques the LS had significantly lower E than did SU, regardless of the depth.

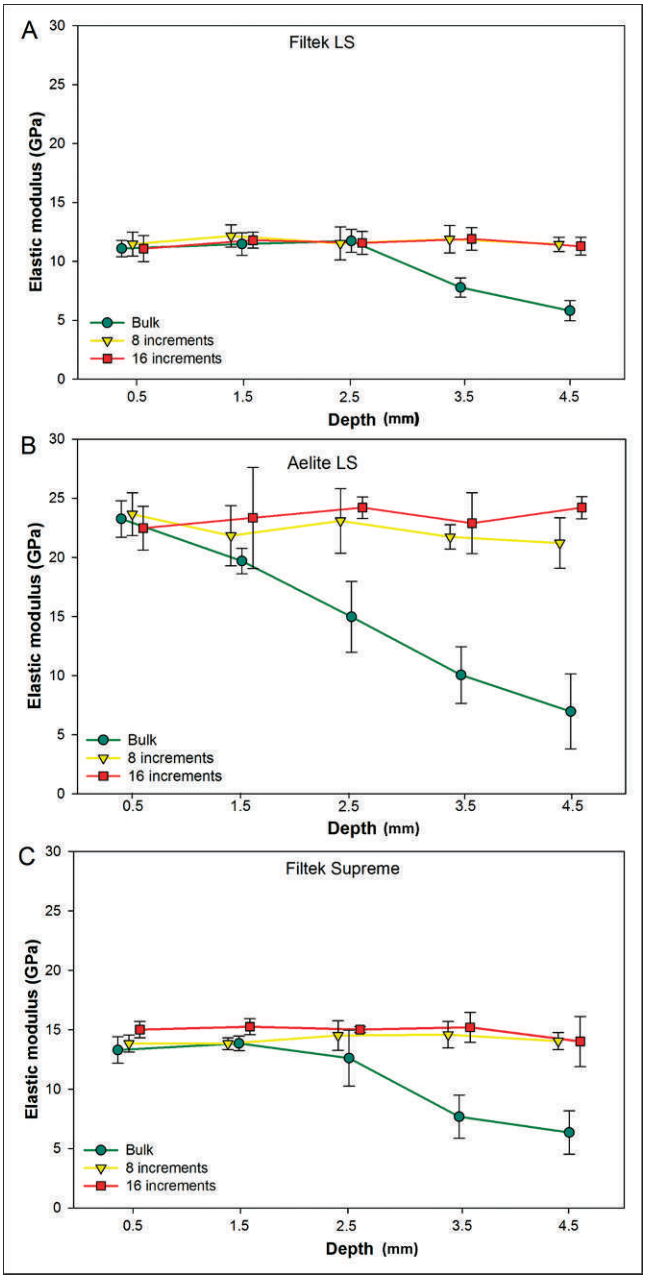

Figure 5. *Elastic modulus at various restoration depths for (A) Filtek LS; (B) Aelite LS; and (C) Filtek Supreme.*

VH

The VH values of the three composite resins for each filling technique at various restoration depths are presented in Figure 6. For the bulk filling technique, the VH of AE and SU decreased when the restoration depth increased, while LS had constant VH up to 2.5 mm and significantly decreased VH afterwards. For the eight-increment technique, LS had constant VH regardless of cavity depth. The VH of AE dropped at 1.5 mm in depth, remained constant from 2.5 to 3.5 mm in depth, and decreased again at 4.5 mm in depth. SU showed a significant reduction in VH at 1.5 and 2.5 mm and remained constant afterwards. For the 16-increment technique, VH remained constant with the increasing restoration depth.

AE and SU had significantly higher VH values than did LS up to 1.5 mm of the restorations for the bulk technique and had significantly higher VH in all depths for the eight- and 16-increment techniques. AE and SU had similar VH values for the bulk and eight-increment filling techniques regardless of the restoration depth. However, AE had significantly higher VH values than did SU up to 3.5 mm in depth for the 16-increment technique.

DISCUSSION

The null hypothesis was rejected: the filling technique and composites significantly affected all properties tested (CS, μTBS, UTS, E, and VH).

Cuspal Deformation

Cuspal deformation in composite restored teeth is affected by many factors, including the size of the cavity, the properties of the restorative material, and the filling technique.[27,28] In this study the size of the cavity was standardized for all samples. Assuming similar tooth properties, shapes, and sizes, the main variables causing differences in cuspal deformation were therefore the properties of the composites and the filling techniques. LS-restored teeth had lower cuspal deformation (statistically significantly so for the incremental fillings), followed by AE and SU, regardless of the filling technique. From a mechanical perspective, the main differences between the three composite resins were the E and polymerization shrinkage. Under certain conditions, cuspal deflection can be expected to increase with increasing E.[29] However, for SU we found higher CS values than for AE, despite its lower E. This result can be explained by the postgel shrinkage, which was lower for Filtek LS compared with Filtek Supreme and Aelite LS. Higher postgel shrinkage values have been shown[30] to increase residual shrinkage stresses and can therefore also increase cuspal deformation of restored teeth.

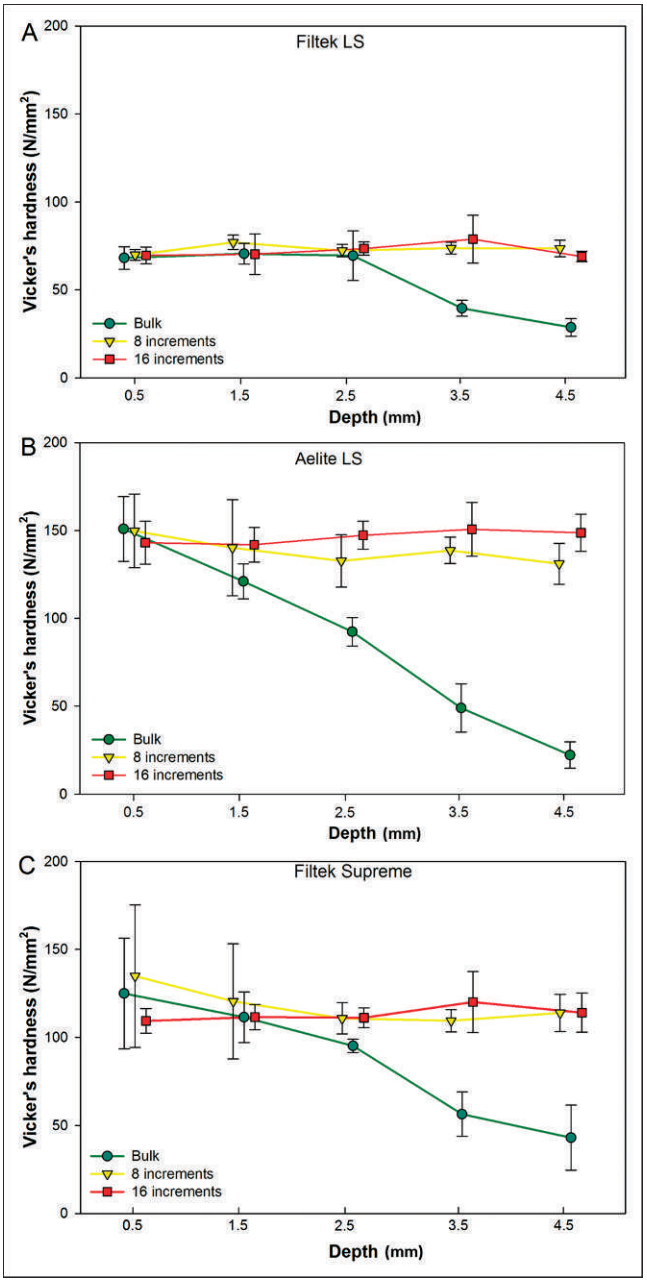

Figure 6. *Vickers Hardness at various restoration depths for (A) Filtek LS; (B) Aelite LS; and (C) Filtek Supreme.*

No significant differences in cuspal deflection between bulk and incremental filling techniques were found when only two or three increments were used in the restorative procedure.[31] In this study, however, we found generally higher cuspal deformation values for AE and SU when the number of increments was eight or 16, compared to the values

found with the bulk filling technique. These successive polymerization steps generated cumulative deformation of the tooth, resulting in a final deformation that exceeded that of the bulk filling technique. We also found that lingual cusps had higher cuspal deformation than buccal cusps, which was statistically significant for Aelite LS and Filtek Supreme when restored with the 16-increment filling technique. This result can be explained by the amount of remaining tooth structure. Third molars had narrower cervical areas lingually than they did buccally, and, thus, the lingual cusps can be expected to be less stiff than the buccal cusps.

μTBS

Although it is important for clinicians to choose restorative materials and filling techniques that cause minimal cuspal deformation, such choices should not come at the expense of the μTBS and the physical properties of the material. Excellent bonding and properties are critical for optimal clinical performance of a restoration. In the present study the μTBS obtained with the incremental techniques was higher than that obtained with the bulk technique in all materials tested. This result is consistent with those of other studies[12,19] and can be attributed to the composite receiving sufficient light energy to properly cure in an incremental technique, whereas the composite in a bulk filling is usually too thick to reach the same degree of cure throughout the entire depth of the restoration.[17] For the AE and SU, both the eight- and 16-increment techniques showed similar μTBS values, which means that the eight-increment technique had the benefit of less cuspal deformation than the 16-increment technique without compromising the μTBS.

μTBS has also been related to polymerization shrinkage stresses, whereby a higher stress condition has been related to lower μTBS values.[32] This study, however, found lower μTBS values for teeth that had less deformation. The lower μTBS might have been caused by differences in composite cure. A lesser cure in the bulk filled restoration would reduce the tensile strength of the composite, as shown in Table 4. Observation of the failure modes also seems to support this explanation, because the bulk filled restorations tended to have significantly higher incidence of mixed failures than did the incrementally cured restorations. Additionally, note that the light emitted by the light-curing unit, which had the tip touching the cusps, for bulk restoration was only 22 J/cm^2, compared to 176 and 352 J/cm^2 with the incremental techniques, which reduced its cure.

Mechanical Properties

The results of this study showed that all composites had similar UTS values for bulk filling regardless of region of the restoration, but the UTS values were significantly lower than the values obtained when the restoration was placed in increments. UTS has been directly correlated with the quality of polymerization.[12] Therefore, considering their equivalent UTS values, both the eight- and 16-increment techniques provided more light energy for better polymerization. It has also been reported[19,33] that UTS of composite decreased when it was cured under constrained conditions. In the current study the UTS values were the lowest in the bulk placed restorations, while the incrementally cured restorations had significantly higher UTS values. Since it can be argued, based on the tooth deformation results, that the incremental restorations were more highly stressed, this study found that UTS increased with increasing shrinkage stress. Differences in UTS values between bulk and incremental techniques may be partly ascribed to differences in the degree of cure, which are reflected in the VH values: at the bottom of the restoration the VH values were lower when using the bulk technique, and, thus, the mechanical properties may not have been fully developed. However, VH values could also not explain why the UTS values of the bulk fillings were similar in the top and bottom regions because significantly lower VH values were found in the bottom region. The VH results could also not explain why the UTS at the top region was lower for bulk than for incrementally placed restorations, because they had similar VH values regardless of filling technique.

All composites showed constant values of VH in all depths of the restorations when 16 increments were used, suggesting adequate polymerization and degree of conversion of the monomers.[33] For the eight-increment technique, AE and SU had decreased VH near the restoration surface, which remained constant and dropped again in the bottom region of the restoration, while the VH of LS remained constant at all depths. For bulk fillings, the VH values of AE and SU gradually decreased with depth, while the VH of LS remained constant to a depth of 2.5 mm before decreasing. The VH values confirmed that a bulk filling technique may not allow adequate polymerization of the monomers deeper in the restoration. We also showed that the E decreased with increasing

restoration depth for all composites that were cured in bulk. SU and LS had constant E values through the depth when filled in eight increments, whereas all materials had constant E when filled in 16 increments. Similar results have been reported[34] in an investigation of the relationship between E and depth of the cavity. It can be expected that the quality of the polymerization throughout a restoration is essential for adequate mechanical properties of a composite material and therefore justifies the need for incremental placement.[35]Monomers such as bisphenol-A glycol dimethacrylate and triethylene glycol dimethacrylate exhibit 5.2% and 12.5% volumetric shrinkage, respectively, but this has been reduced to 2% to 6% in composites as a result of the presence of fillers.[36] An increase in filler volume content leads to reduction in volumetric shrinkage as the resin content is smaller, yet high filler volume results in stiff materials with a high E, which generates higher stresses for the same shrinkage values.[23] This aspect may explain the similar performance of Filtek Supreme and the Aelite LS. Although Aelite LS is marketed as a low-shrink composite, it has higher filler content than does Filtek Supreme.

Filtek LS is a novel low-shrink resin utilizing silorane monomers. The polymerization reaction of this composite is based on a cationic ring-opening polymerization, which results in shrinkage values below 1%.[37] A significant decrease of shrinkage stress has been reported[23] compared to that associated with methacrylate-based composites. This may explain the performance of the Filtek LS restorations compared with other composite resin tested in this study.

The problems frequently observed with posterior composite restorations, usually in the gingival area, could be related to the inferior mechanical properties and insufficient bonding to tooth structures. The finding of this study could be important for clinicians during restorative procedures of large cavities. Increasing the number of increments resulted in higher cuspal deformation, which could potentially lead to fracture of the enamel and postoperative sensitivity. Although it lowered the cuspal deformation, the bulk filling technique did not allow thorough curing throughout the restoration depth and thus compromised the bonding and mechanical properties of the restorative composite. To manage large cavity restorations the use of oblique increments has been reported[23] to result in lower residual stresses than are associated with horizontal increments. Therefore, clinicians must balance the bonding and good material properties with low cuspal deformation using the incremental technique with 2.0-mm increments for the longevity of both dental structures and the large posterior restoration.

CONCLUSIONS

Cuspal deformation in molars restored with composite was higher when 16 increments were used than when an eight-increment technique was used. One of the low-shrink composite resins (Filtek LS) also caused lower cuspal deformation. The μTBS and the composite tensile strength were similar for the eight- and 16-increment techniques and higher than those associated with the bulk filling technique. The physical properties (tensile strength, E, and VH) were approximately constant throughout the restoration depth when filled in eight or 16 increments. Incremental filling was found to be crucial for thorough curing of large restorations, although too many increments are not necessary and might lead to an increase in undesirable tooth deformation.

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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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Incremental Filling Technique and Composite Material—Part II: Shrinkage and Shrinkage Stresses

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Clinical Relevance

Using low-shrink composites applied in medium increment sizes of approximately 2 mm provided the best balance compared to bulk or 1-mm increment placements.

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SUMMARY

Objectives: **Finite element analysis (FEA) was used to study polymerization shrinkage stress in molars restored with composites and to correlate those stresses with experimentally measured tooth deformation.**

Methods: **Three composites (Filtek LS, Aelite LS Posterior, Filtek Supreme) and three filling techniques (bulk, 2.0-mm increments, and 1.0-mm increments) for restoring a molar were simulated in a two-dimensional FEA. Polymerization shrinkage was modeled using post-gel shrinkage, which was measured using the strain gauge technique (n=10). Cuspal tooth deformation, measured at the buccal and lingual surfaces with strain gauges in a laboratory study, was used to validate the analysis. Residual shrinkage stresses were expressed in modified von Mises equivalent stresses. Linear Pearson correlations were determined between the laboratory and FEA results.**

Results: **Post-gel shrinkage values (in volume %) were: Filtek LS (0.11 ± 0.03) < Aelite LS Posterior (0.51 ± 0.02) < Filtek Supreme (0.62 ± 0.09). The 1.0-mm increment filling caused substantially higher stresses and strains in the**

cervical enamel region. Significant correlations were found between: elastic modulus and FEA strain, elastic modulus and FEA stress, post-gel shrinkage and FEA strain, post-gel shrinkage and FEA stress, FEA strain and cuspal deformation by strain gauge, and FEA stress and cuspal deformation by strain gauge ($p<0.05$).

Conclusions: **Increasing the number of increments and high post-gel shrinkage and/or elastic modulus values caused higher stresses in the remaining tooth structure and tooth/restoration interface. Cuspal deformation measured with the strain gauge method validated the finite element analyses.**

INTRODUCTION

Composite resins are widely used in dentistry to restore teeth with structural loss due to their esthetics and physical properties. However, these restoratives have polymerization shrinkage as an inherent problem that may cause residual stresses in the tooth, even when not in function.[1,2] Clinical signs that have been associated with polymerization shrinkage stress include inadequate adaptation at tooth/restoration interface, micro-cracking, postoperative sensitivity, microleakage, and secondary caries.[3,4] These issues are often responsible for replacement of composite restorations in posterior teeth.[5,6] Changes in material formulations and filling techniques, aimed at reducing volumetric contraction and shrinkage stress, have been the primary approaches for reducing the development of residual stresses.[7,8]

Restorative composite formulations have been continuously improved by modifying filler content[9] and monomer types.[10] Silorane-based composites were developed to minimize the polymerization contraction. These monomers are derived from a chemical combination between the components of siloxanes and oxiranes.[11] The polymerization reaction occurs by photo cationic ring opening, and results in a lower polymerization shrinkage compared with resins that are based on methacrylates.[12] Another important physical property that influences the stress development is the elastic modulus, which is also associated with the composition of a material. Elastic modulus has been shown to increase with filler content.[13,14] Since an increase in elastic modulus will increase the stress under the same strain conditions, an increase in elastic modulus tends to increase residual shrinkage stresses. Manufacturers may be tempted to produce new low-shrink composites by reducing the elastic modulus. However, if the elastic modulus is low, the restorative material may not sufficiently recover the structural integrity of the original tooth to support masticatory loads.[14] Composite resins with high elastic modulus produce more rigid restorations, which increase the effect of polymerization contraction on residual shrinkage stresses.[15]

Filling techniques also influence stress distributions. The potential of incremental composite placement technique to reduce the shrinkage deformation and stress at the adhesive interface is controversial.[16-18] An incremental technique could increase shrinkage stresses due to incremental cuspal deformation by each polymerized increment. The incremental cuspal deformation also leads to a reduction in the volume of the cavity, reducing the amount of composite that is placed in subsequent increments.[18]

A previous study, which will be referred to as Part I, examined the effect of composite resin type and filling technique on cuspal deflection, microtensile bond strength, and mechanical properties of the composite resins in class II restorations.[19] It was found that restorative techniques that cured restorations in 8 (2.0-mm) or 16 (1.0-mm) increments instead of bulk increased cuspal strains. However, the influence of these factors on the shrinkage stress inside the composite restoration, tooth structure, or along the adhesive interface could not be determined by the laboratory tests.

The purpose of this study was to evaluate further the outcomes of the experimental results by correlating cuspal deformations for different composite materials and filling techniques with residual shrinkage stresses using finite element analysis (FEA). The post-gel shrinkage values required for the stress analyses were determined experimentally using the strain gauge method, while the elastic modulus values obtained in Part I are used in the shrinkage stress calculations.

MATERIALS AND METHODS

Post-gel Shrinkage Measurements

Composite post-gel linear shrinkage was determined using the strain gauge method.[20] The materials used in this study were two low shrink composite resins, Filtek LS (LS) and Aelite LS Posterior (AE), and one conventional composite resin, Filtek Supreme (SU). Their composition and manufacturer information are listed in Table 1. The composite resin was shaped into a hemisphere and placed on top of a biaxial strain gauge (CEA-06-032WT-120, Measurements

Table 1: *Dental Composites Tested in the Study (Information Provided by the Respective Manufacturers)*

Composite Resins	Wt%	Vol%	Filler Type	Matrix	Manufacturer
Filtek LS	76	55	Quartz and yttrium fluoride (0.1-2.0 μm)	TEGDMA and ECHCPMS	3M ESPE, St Paul, MN, USA
Aelite LS Posterior	84	74	1.1 μm	Bis-GMA and UDMA	BISCO, Schaumburg, IL, USA
Filtek Supreme	82	60	Silica nanofillers (75 nm) zirconia nanofillers (5-10 nm) and agglomerated zirconia/silica nanoclusters (600-1400 nm)	Bis-GMA, Bis-EMA, UDMA, TEGDMA	3M ESPE, St Paul, MN, USA

Abbreviations: Bis-EMA, bisphenol-A hexaethoxylated dimethacrylate; Bis-GMA, bisphenol-A glycol dimethacrylate; ECHCPMS, 3,4-epoxycyclohexylcyclopolymethylsiloxane; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

Group, Raleigh, NC, USA) that measured shrinkage strains in two perpendicular directions. The perpendicular strains were averaged since the material properties were homogeneous and isotropic on a macro scale. The composite was light-cured using a quartz-tungsten-halogen unit (Demetron, Kerr, Orange, CA, USA) with the light tip placed at 1-mm distance from the surface of the composite. The radiant exposure was set at 24 J/cm^2 (600 mW/cm^2 $\times$ 40 seconds). A strain conditioner (ADS0500IP, Lynx Tecnologia Eletrônica, São Paulo, SP, Brazil) converted electrical resistance changes in the strain gauge to voltage changes through a quarter-bridge circuit with an internal reference resistance. Microstrain resulting from polymerization shrinkage was monitored for 10 minutes, starting from the beginning of photoactivation. Ten specimens were tested for each composite. The post-gel shrinkage value at 10 minutes was used in the finite element analysis.

The mean shrinkage strain, which is the linear shrinkage, of the samples (n=10) was converted to percentage and multiplied by three to obtain the volumetric shrinkage. One-way analysis of variance (ANOVA) followed by Tukey honestly significant difference (HSD) post hoc tests (p=0.05) were used for the statistical analysis.

Residual Stress Calculation: Finite Element Analysis

To calculate corresponding residual stress in the tooth, a two-dimensional (2D) finite element simulation was carried out for a mesial-occlusal-distal restoration with the cavity floor in dentin. The geometric model was based on a digitized buccolingual cross section of a third molar with similar dimensions as the teeth selected in laboratory tests of Part I of this study. Coordinates were obtained using ImageJ software (public domain, Java-based image processing and analysis software developed at The National Institutes of Health, Bethesda, MD, USA). Only the cervical portion of the root was simulated since the rest of the root did not affect the coronal stress distribution.[14] A simplified boundary condition was assumed at the cut-plane of the root (fixed zero-displacements in both horizontal and vertical directions). The elastic modulus of enamel was 84 GPa and Poisson's ratio 0.30; the dentin elastic modulus was 18 GPa and the Poisson's ratio 0.23.[21] The elastic modulus values of the three composites filled by the three techniques at five restoration depths were obtained in Part I of this study. They ranged from 5-11 GPa, 5-24 GPa, and 6-15 GPa for Filtek LS, Aelite LS Posterior, and Filtek Supreme, respectively. The Poisson's ratio was chosen to be the same for all composites at 0.24.[21]

The finite element analysis was performed using MSC.Mentat (preprocessor and postprocessor) and MSC.Marc (solver) software (MSC Software Corporation, Santa Ana, CA, USA). The total number of FEA models was nine for the three different filling techniques (bulk filling, 2.0-mm increments and 1.0-mm increments) and the three composites (Filtek LS, Aelite LS Posterior, and Filtek Supreme). A plane strain condition was assumed for the tooth cross sections. Due to this 2D strain condition and consequently 2D finite element model, no distinction was made between the mesial and distal increments. The 2.0-mm and 1.0-mm increment techniques of the experimental study (Part I) were therefore simulated in 4-mm and 8-mm increments, respectively. Polymerization shrinkage was simulated by thermal analogy. Temperature was reduced by 1°C, while the linear shrinkage value (post-gel shrinkage) was entered as the coefficient of linear thermal expansion.

Modified von Mises equivalent stress was used to express the stress conditions, using compressive-tensile strength ratios of 37.3, 3.0, and 6.25 for the enamel, dentin, and composite, respectively.[22] Stress values were recorded in the integration points of each element and in nodes along material interfaces at either aspect (tooth and restoration). Linear

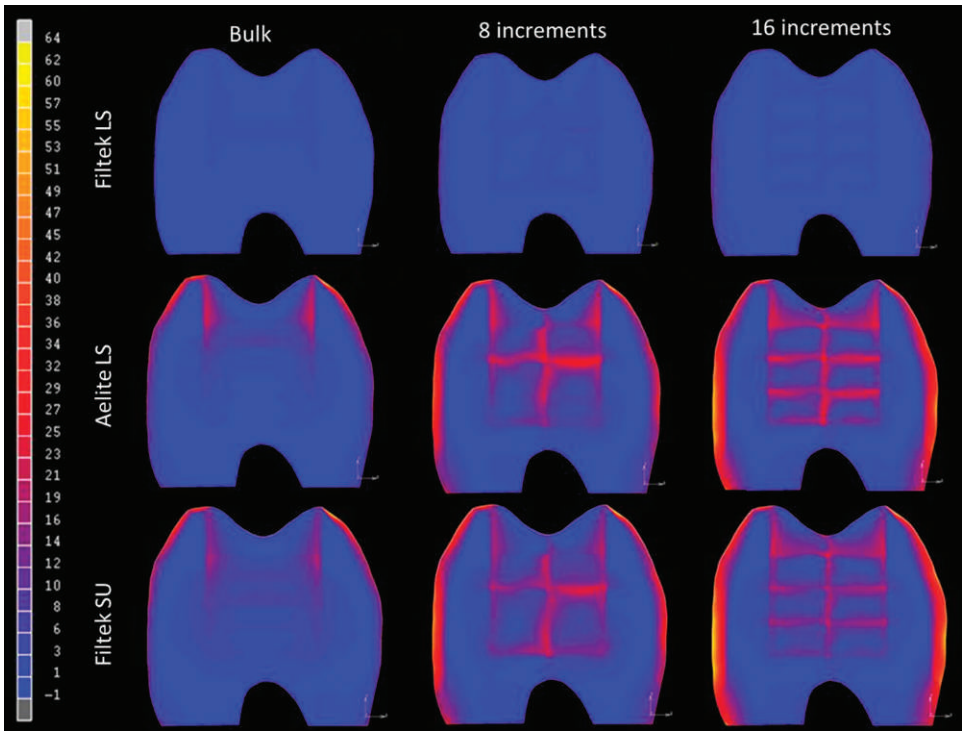

Figure 1. *Stress distributions calculated by finite element analysis (modified von Mises equivalent stresses, MPa).*

Pearson correlation tests were performed between elastic modulus, post-gel shrinkage, experimental bond strength, cuspal deformation determined experimentally and by FEA, stress in enamel, and stress along the restoration interface ($p<0.05$). The mean values of the 5% highest stress and strain were determined for the cervical enamel (where the strain gauges were fixed in laboratory tests). These values were used to calculate Pearson correlations with cuspal deformation by strain gauges. Furthermore, strain values were obtained at nodes of the buccal external surface corresponding to the same position where the strain gage was fixed in laboratorial tests. The stress values at the interface between composite resin and dentin were obtained corresponding to five depths of the restoration in 2D model and correlated with elastic modulus and post-gel shrinkage values in the five depths of the restoration of laboratorial tests. The mean values of the 5% highest stresses were determined for the dentin/composite interface and correlated with microtensile bond strength values.

RESULTS

Post-gel Shrinkage

The mean values and standard deviations for the post-gel shrinkage of three composites are presented in Table 2. One-way ANOVA revealed statistical difference among the composites ($p<0.001$). Filtek Supreme had the highest mean volumetric shrinkage value and Filtek LS had the lowest value.

Finite Element Analysis

Stress distributions for all groups are shown in Figure 1 and values of stress and strain obtained by finite element analysis for buccal cuspal and lingual cuspal are shown in Table 3. Compared to the other two composite resins, the Filtek LS restored teeth were least influenced by filling technique. The bulk filling technique resulted in lower stresses at the enamel/composite interface than the 2.0-mm increment technique, and the 2.0-mm techniques was lower than the 1.0-mm increment technique for Filtek Supreme and Aelite LS Posterior. The Aelite LS Posterior composite resulted in the highest stress values, while Filtek LS generated the lowest stress values along both enamel and dentin interfaces, irrespective of restorative technique. The 1.0-mm increment technique resulted in a substantial stress

Table 2: *Mean (SD) Volumetric Post-gel Shrinkage**

Composite Resins	Volumetric Post-gel Shrinkage,%
Filtek LS	0.11 (0.03)^{A}
Aelite LS Posterior	0.51 (0.02)^{B}
Filtek Supreme	0.62 (0.09)^{C}

** Different uppercase letters indicate significant difference between the composites (p<0.05).*

Table 3: *Values of Stress and Strain Obtained by Finite Element Analysis (FEA) for Buccal Cuspal (B) and Lingual Cuspal (L)*

Composite Resin	Bulk Filling						8 Increments						16 Increments					
	FEA Stress		FEA Strain		FEA Interface		FEA Stress		FEA Strain		FEA Interface		FEA Stress		FEA Strain		FEA Interface	
Depth	(B)	(L)	(B)	(L)	(B)	(L)	(B)	(L)	(B)	(L)	(B)	(L)	(B)	(L)	(B)	(L)	(B)	(L)
Filtek LS																		
4.5	3.7	4.1	21.8	21.1	1.5	1.53	3.5	4.96	20.91	25.97	1.46	1.66	5.14	4.8	30.63	24.79	1.56	3.7
3.5	3.7	3.4	23.4	24.9	1.3	1.31	5.12	4.79	31.75	35.46	1.53	1.52	7.32	4.93	46.49	36.85	2.00	3.7
2.5	2.1	3.1	15.8	22.9	1.7	1.64	4.36	7.75	34.07	60.05	1.73	1.66	5.51	8.09	43.85	63.13	1.58	2.1
1.5	1.7	2.1	13.1	15.8	1.9	1.84	5.27	8.84	42.67	69.88	2.26	1.95	7.06	9.54	58.09	75.96	1.70	1.7
0.5	1.8	1.9	13.6	14.8	1.3	1.31	5.85	8.42	46.04	67.84	1.56	1.47	9.25	10.32	73.49	83.49	2.03	1.8
Aelite LS Posterior																		
4.5	21.8	23.5	131.8	124.7	13.4	13.71	18.96	29.21	113.05	153.9	13.28	14.53	30.82	29.38	183.65	152.19	12.35	21.8
3.5	20.4	17.9	130.8	132.2	14.0	13.67	27.18	26.82	167.79	198.78	14.88	14.54	43.22	29.29	274.88	219.65	15.61	20.4
2.5	11.8	18.2	92.2	138.6	11.2	10.47	23.24	42.06	181.42	325.83	11.47	10.86	32.46	47.91	258.61	374.61	11.56	11.8
1.5	10.7	14.2	85.9	111.0	5.7	5.56	26.98	45.71	217.87	361.51	17.12	14.84	41.32	56.15	340.03	447.77	14.93	10.7
0.5	11.1	11.7	87.2	93.9	3.7	3.5	28	40.42	220.68	326.2	10.21	9.40	52.51	58.84	417.72	476.91	13.74	11.1
Filtek Supreme																		
4.5	22.8	24.9	136.1	129.6	9.3	9.74	21.57	30.87	128.68	161.96	10.25	11.53	34.23	32.22	203.83	166.49	11.12	22.8
3.5	23.3	20.9	147.3	152.3	8.8	8.62	31.09	29.24	192.62	216.55	11.02	10.90	48.15	32.66	306.28	244.45	14.45	23.3
2.5	13.4	19.9	103.4	150.3	11.3	10.73	26.28	46.72	205.21	361.71	11.01	10.55	35.69	52.74	284.21	411.81	12.50	13.4
1.5	11.6	14.5	90.8	111.2	8.5	8.34	31.14	52.42	251.41	414.38	14.48	12.15	44.74	60.98	367.95	485.81	12.44	11.6
0.5	11.6	12.0	88.8	94.7	5.78	5.67	33.38	48.86	262.51	393.63	10.06	9.21	57.06	63.86	453.62	517.17	10.54	11.6

increase at the external tooth surface, particularly in the cervical enamel region, compared to the bulk and 2.0-mm increment techniques.

Correlations Between Experimental and FEA Results

Figures 2 and 3 compare cuspal deformation obtained with strain gauges in the laboratory study and strains obtained with the FEA in the same region of enamel on buccal and lingual surfaces. Cuspal deformation values from the laboratory study were very similar to the deformation values calculated by FEA (r=0.946).

The values of strain and stress by finite element models and Pearson correlations between various parameters are shown in Table 4. Significant linear correlations were found among the following parameters ($p<0.05$): elastic modulus values and FEA strain, elastic modulus and FEA stress, post-gel shrinkage and FEA strain, post-gel shrinkage and FEA stress, FEA strain and FEA stress, cuspal strain measured by strain gauges and FEA strains, and cuspal strain measured by strain gauges and FEA stress. No significant correlations were found between elastic modulus and cuspal deformation measured by strain gauge, post-gel shrinkage and cuspal deformation measured by strain gauge, and FEA stresses along the interface and the microtensile bond strengths. The stress values used were the 5% stress, cervical stress, and top/bottom stress.

DISCUSSION

The results of the present study confirmed that the magnitude and distribution of residual shrinkage stresses in the restoration/tooth complex depended on the composite material's post-gel shrinkage and elastic modulus as well as the filling technique used.

To calculate the shrinkage stresses, polymerization shrinkage behavior must be modeled. Since not all shrinkage generates stresses, a "post-gel" shrinkage value was used in the analysis. Post-gel shrinkage was defined as the portion of the total polymerization shrinkage that causes stresses, and was measured using the strain gauge technique.[23] The post-gel shrinkage of Filtek LS (0.11% by volume) was almost five times lower than that of Aelite LS Posterior (0.51%) and six times lower than that of Filtek Supreme (0.62. The low post-gel shrinkage values of Filtek LS could be explained by the silorane molecules, which have a siloxane core with four oxirane rings attached, that open during polymerization to link to other monomers. The oxirane ring opening causes volumetric expansion that partially compensates the shrinkage from

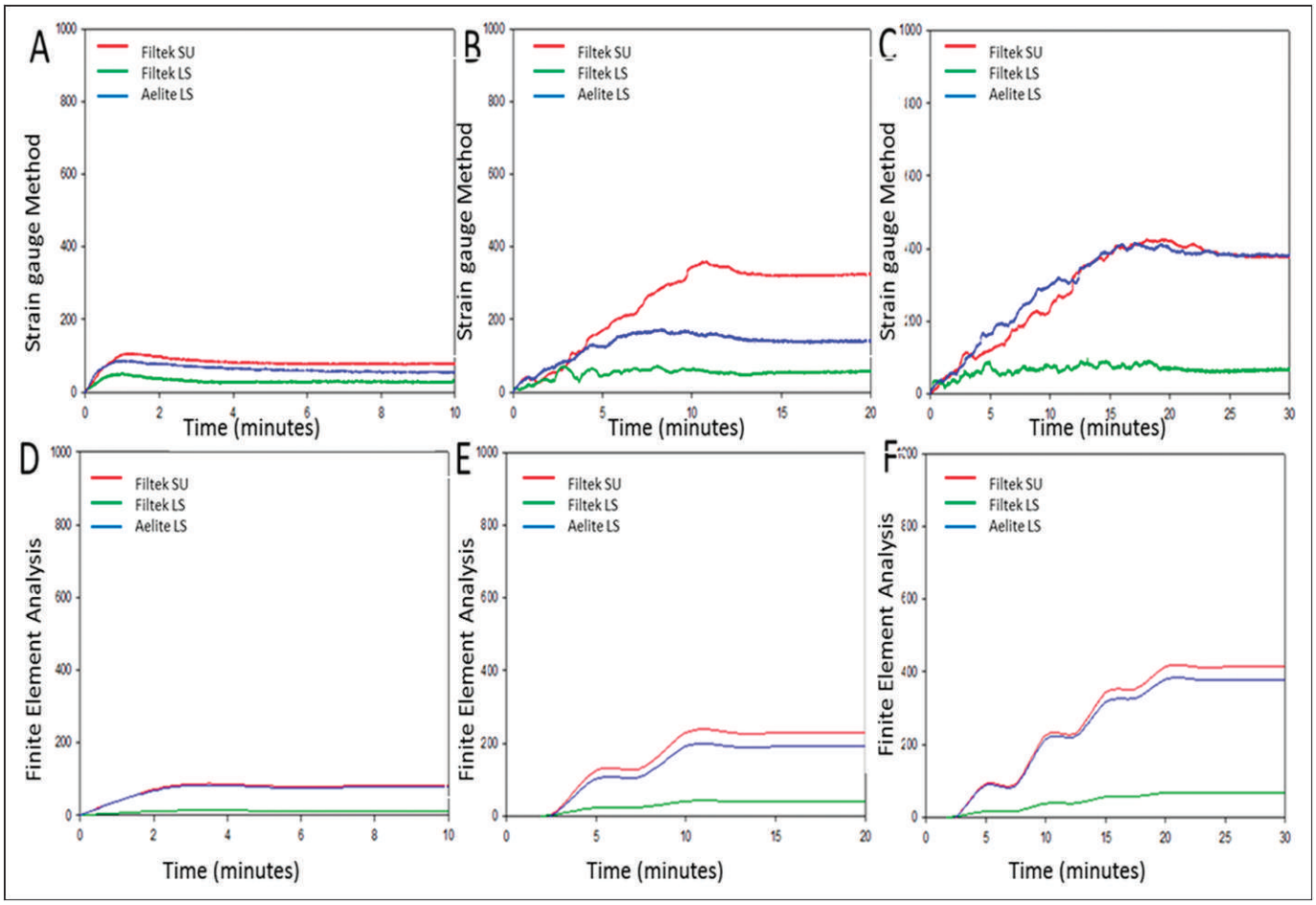

Figure 2. *Cuspal deformation at buccal cusp. (A): Bulk filling, strain gauge method. (B): 8 increments, strain gauge method. (C): 16 increments, strain gauge method. (D): Bulk filling, finite element analysis. (E): 8 increments, finite element analysis. (F): 16 increments, finite element analysis. A, B, and C are data from the laboratory study in Part I of this study.*[19]

molecular union.[12,24] Although both Aelite LS Posterior and Filtek Supreme have a Bis-GMA–based matrix, the Aelite LS Posterior had a lower post-gel shrinkage value than Filtek Supreme. This may be due to the higher filler loading of Aelite LS Posterior. A higher filler content means less volume of the resin matrix, and thus less composite shrinkage.[25] However, before interpreting this lower shrinkage to mean a lower shrinkage stress, it is important to also take into account that increasing filler content generally results in an increased elastic modulus,[25] and thus higher rigidity of a restoration, which in turn tends to increase shrinkage stresses.

Shrinkage stress is thus not only determined by the polymerization shrinkage, but is dependent on the combination of physical properties as well as structural features. Part I of this study covered the experimental components. Experimental tests are fundamental for the assessments of dental structures and restorative materials because they already naturally combine all relevant factors that determine a mechanical response to polymerization shrinkage. However, experimental studies also have limitations, such as their inability to obtain information about the internal behavior of a restoration and the determination of stresses. FEA was developed as an engineering tool to solve stress-strain conditions in complex structures while taking into account the interaction of the various factors, including linear and nonlinear effects caused by deformations and material properties. FEA has become a vital element in any comprehensive evaluation of complex stress conditions inside restored teeth.[3] This study used 2D analysis to study the stress distributions and deformations by assuming a plane strain condition in the tooth structure. This means that we could calculate the three-dimensional (3D) stress condition in a 2D geometric model. Simplification into a 2D geometry has the advantage of immediate insight and relatively

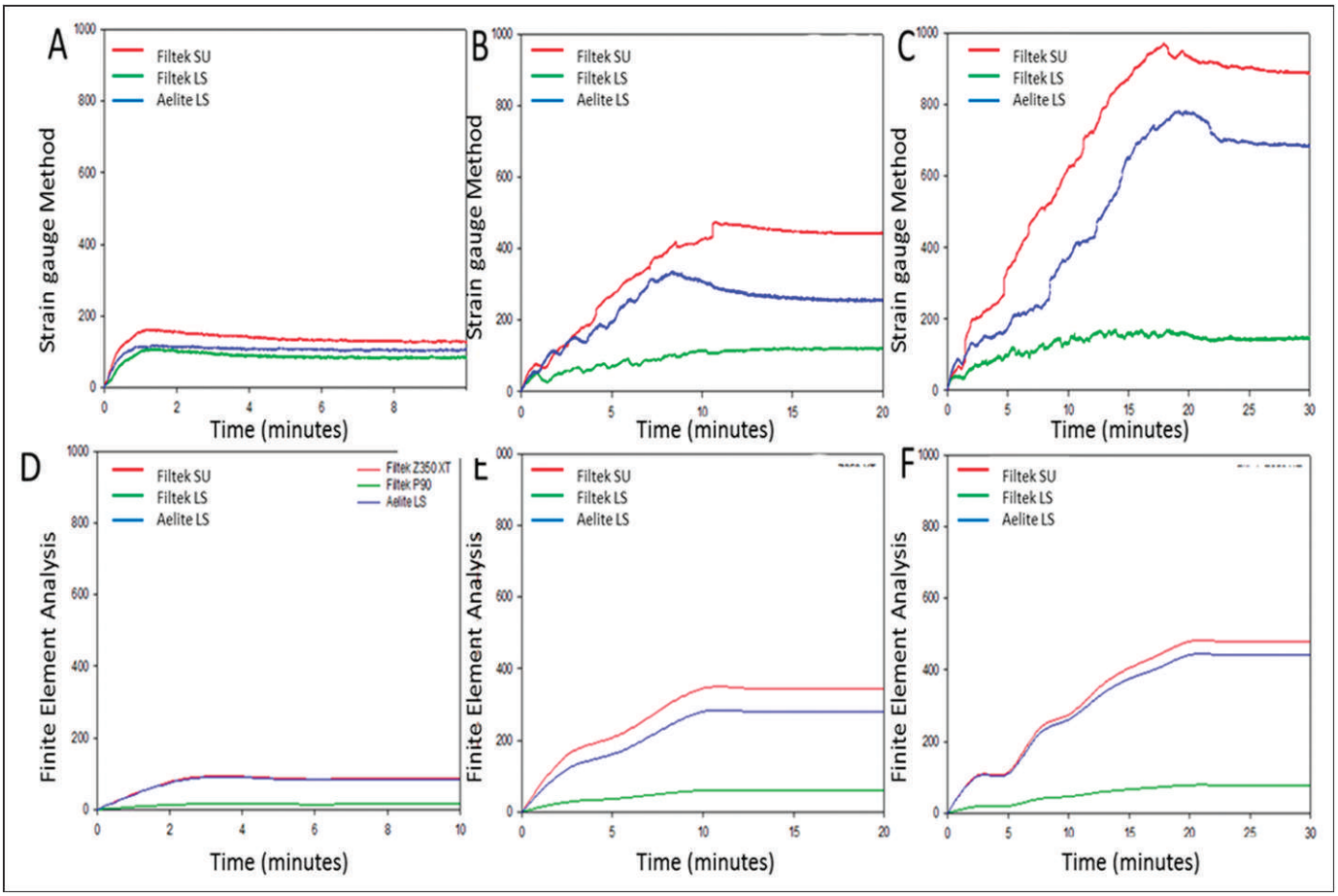

Figure 3. *Cuspal deformation at lingual cusp. (A): Bulk filling, strain gauge method. (B): 8 increments, strain gauge method. (C): 16 increments, strain gauge method. (D): Bulk filling, finite element analysis. (E): 8 increments, finite element analysis. (F): 16 increments, finite element analysis. A, B, and C are data from the laboratory study in Part I of this study.*[19]

affordable operating costs in modeling and analysis time, while the definition of the stress conditions can still provide significant insight into the 3D stress state. Stresses in three dimensions were integrated into one scalar value by using a modified von Mises criterion to represent the overall stress condition that could be used to show areas with most critical stress concentrations.[26] To ensure that the geometric

Table 4: *Pearson Correlations*

Correlations	*r*	*p*
Elastic modulus[a] × FEA strain	0.726*	0.027
Elastic modulus[a] × FEA stress	0.721*	0.028
Elastic modulus[a] × cuspal deformation by strain gauge[a]	0.567	0.111
Post-gel shrinkage × FEA strain	0.780*	0.013
Post-gel shrinkage × FEA stress	0.798*	0.010
Post-gel shrinkage × cuspal deformation by strain gauge[a]	0.616	0.077
FEA strain × cuspal deformation by strain gauge[a]	0.946*	0.000
FEA stress × cuspal deformation by strain gauge[a]	0.978*	0.000
FEA stress along interface × microtensile bond strength[a]	0.194	0.441

Abbreviations: FEA, finite element analysis.
[a] Values determined in Part I of this study.[19]
** Significant correlations between study factors ($p<0.05$).*

(plane strain) simplification was justified, we validated the FEA results with strain gauge experiments. The validation confirmed that despite the simplifying assumptions, the general response of the FEA models was realistic. The comparison showed a high correlation between the strains calculated by FEA and measured by strain gauges (0.946), and between the stress calculated by the FEA and the strain measured by strain gauges (0.978). These findings validated our 2D analysis.

The validity of our assumptions about the material properties, tooth and cavity geometry, and artificial constraints in the simulation of the restored teeth was tested by comparing the FEA strain outcomes with the experimental strain results obtained in Part I of this study. Although the calculated stresses could not be validated directly from the laboratory experiments, they could be verified indirectly from the deformation and its consequences.[3] In our study, cuspal strains calculated by the FEA were similar to the cuspal strain data collected experimentally using strain gauges placed on cuspal surfaces (Figures 2 and 3). This close similarity supports the validity of our FEA models and stress results.[26]

In the FEA results (Figure 1), among the three composites, the tooth restored with Filtek LS showed the lowest stress concentrations for all restorative techniques. The performance of Filtek LS can be attributed to its low elastic modulus and low post-gel shrinkage. The low post-gel shrinkage demonstrated by Filtek LS is desirable. On the other hand, a low elastic modulus may be indicative of higher wear rates in areas subjected to masticatory forces.[27,28] Aelite LS Posterior (marketed as a low-shrink composite) and Filtek Supreme (which can be considered conventional with respect to polymerization shrinkage) showed stress concentrations at the base of the cusps. Although the post-gel shrinkage of Aelite LS Posterior was 18% lower than that of the Filtek Supreme, its higher elastic modulus (up to 60%) resulted in similar residual shrinkage stresses. The higher elastic modulus of Aelite LS Posterior can be attributed to its higher filler volume compared to Filtek Supreme.[14] The effect of this balance between post-gel shrinkage and elastic modulus may explain the similar performance of Filtek Supreme and Aelite LS Posterior. These results were consistent with values reported by Boaro and others.[25] Reduction of the number of the increments by using a larger volume of composite in each increment resulted in lower residual shrinkage stresses and lower cuspal deformation strains. This restorative protocol may reduce clinically undesirable effects of shrinkage stress, such as cracks, debonding, and postoperative sensitivity. According to Part I of this study, a decrease in the number of increments from 1.0 mm to 2.0 mm did not compromise the bonding performance or the quality of the polymerization, and could thus improve the overall conditions for the clinical longevity of the restoration.

All stresses and strains that the FEA calculated in the tooth and composite materials showed a significant positive correlation with elastic modulus, post-gel shrinkage, and the measured cuspal deformation. Other studies also found that stresses generated by polymerization increase with increasing composite elastic modulus.[10,27,28] The elastic modulus is an important material property that describes the relationship between stress and strain. Materials with high elastic modulus deform less when they are stressed. Thus, when polymerization contraction ("deformation") is restricted by bonding to the cavity walls, a composite with a high elastic modulus will result in higher shrinkage stress than if the composite would have had a low elastic modulus. This effect can be observed in experimental studies where high-compliance devices tend to overestimate the shrinkage stresses.[28-30] The positive correlation between stress and post-gel shrinkage was also consistent with reports in other studies.[23] Weaker correlations were found between the strains measured by the strain gauges and the elastic modulus or post-gel shrinkage values. This might be due to the cumulative effect of experimental variations, which were less when involving FEA analysis. We found no correlation between stress values along the tooth/composite interface determined by the FEA and the bond strength measured by microtensile tests at different cavity depths. This may not be unexpected because the FEA determined the stresses at the interface, not the strength at the interface. Strength is a property that is likely affected by multiple factors. Often shrinkage stress is considered to be one of those factors.[31-34] However, no evidence was found in this analysis, while the results of Part I also showed no statistically significant differences in bond strengths between composites with different post-gel shrinkage values.

This two-part study showed that the quality of bond and mechanical properties can be challenged in large composite restorations by compromised curing, high cuspal deflection, and unfavorable stress distribution in the remaining tooth structures. To select the composite resin material and filling technique for restoring large cavities, clinicians should try to combine good mechanical properties with lower

shrinkage stress. Bulk filling is not a favorable technique to restore large cavities because it may negatively affect the cure and therefore reduce mechanical properties. On the other hand, a 16 increment technique (1-mm) would cause higher residual shrinkage stresses and higher cuspal strains. The cusp flexure results in Part I of this study and the stress analysis performed in Part II demonstrated that an incremental technique using larger rather than smaller increments (approximately 2 mm high) may provide the best balance between adequate mechanical properties, represented by elastic modulus, Vickers hardness, bond strength, ultimate strength, and lower residual shrinkage stresses.

Although FEA was essential to assess the stress conditions, the validity of stress calculations depends on the correct input of material properties, anatomic shape, and restraints of the restored tooth structure. Most of these input variables must be obtained from laboratory tests. Since laboratory tests are often unable to provide all needed input variables for the finite element analysis, which then have to be estimated, simulation experiments remain necessary to validate the stress calculations. A validated finite element model can be further used to predict mechanical failures or investigate questions that cannot be accessed as well in laboratory tests.[16]

CONCLUSIONS

When restoring a tooth with large cavity, increasing the number of increments and using materials with high post-gel shrinkage and elastic modulus values resulted in higher stresses in the remaining tooth structure and at the tooth/restoration interface. The combination of a low post-gel shrinkage composite and a technique that appropriately polymerized the restoration, such as increments that are large enough but not exceeding 2-mm thickness, can minimize the negative effects of residual shrinkage stresses without impairing the mechanical properties of composites. Cuspal strain measured by strain gauges validated and was validated by the finite element analysis. The validation and correlation of experimental and computational methods is an important step in a comprehensive research approach and is essential to justify conclusions drawn from *in vitro* analyses.

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Conflict of Interest

The authors 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 Effect of Light-curing Access and Different Resin Cements on Apical Bond Strength of Fiber Posts

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Clinical Relevance

Cementation of fiber glass posts with self-adhesive cement (RelyX U100) is more predictable than cementation with resin cement using a three-step etch-and-rinse adhesive system (RelyX ARC/SBMP) as its bond strength to apical dentin was not influenced by the level of light-curing access.

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SUMMARY

Purpose: This study evaluated the effect of light-curing access on the bond strength of fiber glass posts to the apical area of bovine roots using self-adhesive cement or dual-cured cement with an etch-and-rinse adhesive system.

Materials and Methods: The root canals of 60 bovine teeth were endodontically treated and filled. A 15-mm-length post space was prepared and roots were randomly divided into three groups, simulating the levels of light-curing access: coronal (C), with 15-mm post space; middle (M), in which the coronal thirds of roots were cut out, leaving a 10-mm post space; and apical (A), in which the coronal and middle thirds of roots were cut out, leaving a 5-mm post space. Fiber glass posts (Reforpost # 3, Angelus) were cemented with RelyX U100 (3M ESPE) or RelyX ARC/Scotchbond Multi Purpose Plus (SBMP) (3M ESPE) (n=10) and light-cured. After 24 hours, the apical thirds of roots were sectioned perpendicularly to the long axis and submitted to a push-out test (0.5 mm/

min, 200 N). The Kruskal-Wallis test compared the three levels of light-curing access, and the Mann-Whitney test compared the cements.

Results: The bond strength was significantly higher in the groups C (p=0.028) and M (p=0.016) when U100 was used, whereas it was similar for both cements in group A. The bond strengths of posts cemented with ARC/SBMP were significantly higher in group A compared to group C (p=0.031).

Conclusions: The type of cement used and the light-curing access level influenced the bond strength between glass fiber posts and root canals. The bond strength of the RelyX ARC/SBMP cement proved to be more dependent on photoactivation than was the RelyX U100 cement. The light-curing access level did not influence the apical bond strength of RelyX U100.

INTRODUCTION

Restoration of endodontically treated teeth exhibiting large coronal loss requires the use of post systems. Resin cements and fiber posts are good choices for such types of treatment.[1] In general, the results of clinical studies have been favorable with regard to the use of fiber posts. However, clinical studies[2-4] have shown post fractures and de-cementation to represent the most frequent types of failure observed.

Several factors may influence the bond strength of root canal posts, including canal depth, the type of resin cement used, and the dentinal substrate. The canal depth hinders access to operatory instruments as well as light transmission through the canal, and the use of translucent fiber posts has not improved light transmission to most apical areas.[5,6] The light intensity inside the canals is insufficient to ensure photoactivation of dual-cure resin cements.[7,8] Such resin cements exhibit lower degrees of conversion of monomers to polymers when photoactivation is not performed.[9-11] In addition, different resin cements exhibit different microhardness values at different root canal depths.[11]

Likewise, the dentinal substrate varies as a function of root canal depth. The density and diameter of the dentinal tubules decrease from the cervical to the apical areas,[12-14] but the amounts of fibrodentin[15] and secondary dentin increase in the apical areas.[16] Techniques for post cementation based on resin infiltration in the interior dentinal tubules are more sensitive and less predictable in the apical area.[14,16]

Although several studies[17-26] have shown that resin cement bond strengths inside the apical third of the post space preparations are lower than those at the cervical third, some authors[27-29] were unable to confirm this difference. Thus, it is important to investigate the factors influencing bond strength of resin cements in the apical third of the post space preparation, emphasizing the role of photoactivation and dentinal substrate in that root canal area.

The aim of the present study was to investigate the effect of light-curing access on bond strength in the apical third of glass fiber posts cemented with a self-adhesive cement system and a conventional dual-cure system (resin cement plus three-step etch-and-rinse adhesive system). The investigated null hypothesis is that the bond strength at the apical third is not affected by the location at which the light is applied or by the type of cement used.

MATERIALS AND METHODS

Root Preparation

A total of 60 bovine teeth were selected and stored in distilled water under refrigeration for up to three months. The roots were sectioned at the cement-enamel junction using a silicon carbide disc (Dentorium, New York, NY, USA), under constant irrigation with water, until 19-mm root specimens were obtained. Endodontic preparation was performed with working lengths up to 1 mm from the apical foramen using rotary instruments (Xmart, Dentsply, Petrópolis, RJ, Brazil) and Easy Pro-design files (Easy, Belo Horizonte, MG, Brazil) by the crown-down technique under irrigation with 2.5% sodium hypochlorite. Next, a final irrigation was performed with trisodium ethylenediamine tetraacetic acid (Biodinamica, Ibiporã, PR, Brazil) for three minutes, and the root canals were rinsed under water and dried using absorbent paper points. The teeth were obturated by the thermoplastic technique, using gutta-percha cones and AH Plus cement (Dentsply), and were stored in water for seven days.

Post Space Preparation

The root canals were prepared for placement of glass fiber post Reforpost #3 (Ângelus, Londrina, PR, Brazil) using Largo burs #2 to #5 (Maillefer-Dentsply, Petrópolis, RJ, Brazil) with a slow rotary

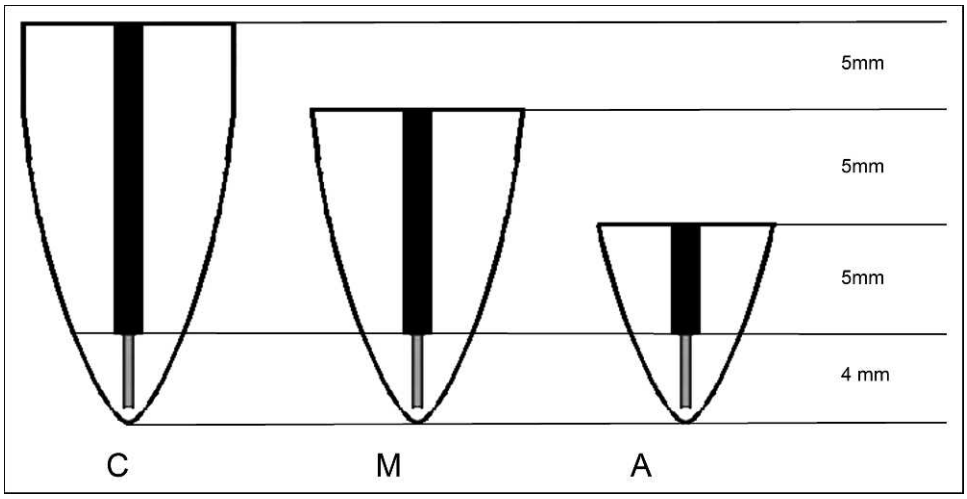

Figure 1. *Classification of roots according to the light-curing access level. C, coronal group: 15 mm; M, middle group: 10 mm; and A, apical group: 5 mm.*

speed and 15 mm of length, leaving 4 mm of apical canal filled.

The roots were then randomly separated into three groups comprised of 20 specimens each, as follows: 1) the coronal access group (group C), which exhibited 15 mm of prepared root canal; 2) the middle access group (group M), in which 5 mm corresponding to the cervical third were removed using a carborundum disc under abundant water irrigation, leaving a 10-mm length of post space preparation; and 3) the apical access group (group A), in which 10 mm corresponding to the cervical and middle thirds were removed, leaving a 5-mm length of post space preparation (Figure 1). Before the posts were cemented, the external root surfaces were covered with black adhesive tape to protect the roots from external light interference.

Post Cementation

Each post was cleaned with 32% phosphoric acid (Uni-etch, Bisco, Schaumburg, IL, USA) for 30 seconds, rinsed with water, and dried using air spray. Two different resin cements were used for cementation (n=10): RelyX ARC dual resin cement with a chemically activated polymerization adhesive system SBMP (ARC/SBMP; 3M ESPE, St Paul, MN, USA) and self-adhesive resin cement RelyX U100 (U100; 3M ESPE). The compositions and manufacturers of the cements are described in Table 1.

For application of ARC/SBMP, dentin was etched with 32% phosphoric acid, Uni-etch, for 15 seconds, rinsed under water, aspirated with endodontic cannulae, and dried with absorbent paper points. The activator was applied using a microbrush, and the excess was removed. Subsequently, the same procedure was performed with the primer and the catalyst. The cement was mixed and inserted into the root canal using a Lentulo bur (Maillefer-Dentsply). The posts were inserted, and a 10-N static load was applied on them during the resin cement photoactivation with a light-emitting diode at 1340 mW/mm^2 (Bluephase, Ivoclar Vivadent, Liechtenstein) for 40 seconds .

For the application of U100, the root canals were rinsed with water, aspirated with endodontic cannulae, and dried with absorbent paper points. The cement was mixed and inserted into the root canal using a Lentulo bur. The posts were then inserted, and the resin cement was photoactivated as described above for ARC/SBMP. The specimens were stored in water for 24 hours and then the push-out test was performed for the apical third of the roots.

Preparation for the Push-out Test

The cemented specimens were transversely sectioned 8.5 mm from the apex with a diamond disc (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling. Two slices that were approximately 1 mm thick were obtained (Figure 2). The thickness of each slice was measured using a digital caliper (Mitutoyo Digimatic Caliper Serie 500, Mitutoyo Sul Americana, Suzano, SP, Brazil), and the posts were subjected to the mechanical test using a plunger with a 1-mm-diameter tip (placed in contact only with the posts). The load was applied to the most apical face of each slice, which was mounted in an

Table 1: *Compositions, Lot Numbers, and Manufacturers of the Resin Cement Systems*

Resin Cement Systems	Composition	Manufacturer
RelyX ARC/Adper Scotchbond Multi-plus	Silicon-treated ceramic, triethylene glycol dimethacrylate (TEGDMA), bisphenol A diglycidyl methacrylate (Bis-GMA); silicon-treated silica, functionalized dimethacrylate polymer: Lot No. FX8HW	3M ESPE
	Activator: ethylic alcohol, sodium benzenesulfinate: Lot No. 8LA	
	Primer: water, 2-hydroxyethyl methacrylate, polycarboxylic acid co-polymer: Lot No. 8BU	
	Catalyst: (1-methyl ethylidene) bis[4,1-phenylene oxi (2-hydroxy-3,1,-propanediyl)] bis methacrylate, 2-hydroxyethyl methacrylate, benzoyl peroxide: Lot No. 8BE	
RelyX U100	Base: glass fiber, methacrylate phosphoric acid esters, triethylene glycol dimethacrylate, silane-treated silica, sodium persulfate	3M ESPE
	Catalyst: glass fiber, substitute dimethacrylate, silane-treated silica, sodium *p*-toluenesulfonate, calcium hydroxide: Lot No. 415462	

apical to coronal direction, using a universal test machine (Emic DL 3000, Emic, São José dos Pinhais, PR, Brazil) with a 200-N load cell (CCE200N, Emic) and a speed of 0.5 mm/min. The maximal extrusion load (Newtons) was recorded (Tesc Version 3.05, Emic). To express the bond strength in MPa, the load was divided by the bond interface area obtained according to the following equation:

$$A = 2\pi rh,$$

where π is the constant 3.14, r is the post radius, and h is the slice thickness in millimeters.

Analysis of Failure

Following the push-out test, each specimen was photographed using a stereomicroscope (Zeiss, Jena, Oberkochen, Germany) at 40× magnification and the images were examined to establish the modes of failure. The modes of failure were assessed by two independent calibrated examiners (kappa=0.72; 95% CI=0.62-0.82) and classified as follows: 1) adhesive failure between dentin and cement; 2) adhesive failure between post and cement; 3) cement cohesive failure; and 4) mixed failure. Interexaminer disagreements were solved by consensus.

Statistical Analysis

The Kolmogorov-Smirnov test showed that the data did not assume a normal distribution (F=0.137; p=0.007), and the Levene test did not identify differences between variances (F=1.979; p=0.162). The nonparametric Kruskal-Wallis test was used to evaluate the effect of the light-curing access on the bond strength of the cements. The Mann-Whitney test was used to investigate differences between the two cements. Statistical analysis was performed using SPSS version 16.0 software (SPSS, Chicago, IL, USA) with a significance level of $\alpha = 0.05$ used in all tests.

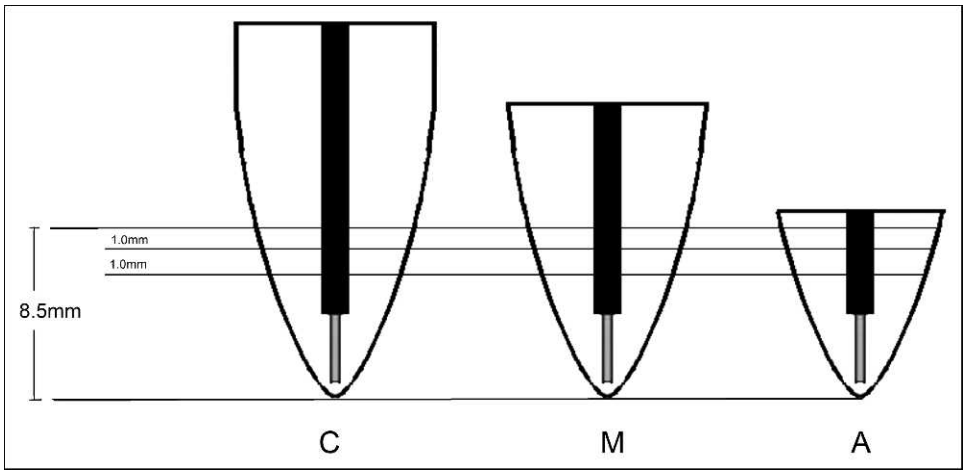

Figure 2. *Illustration of the root sections obtained from each group for the push-out test. C, coronal group; M, middle group; and A, apical group.*

Table 2: *Means (Standard Deviations) of Push-out Bond Strength in the Apical Third of Root Space Preparation (MPa) (n=10)*[a]

Light-curing Access	Resin Cement System	
	Mean (SD)	
	U100	ARC/SBMP
Coronal	8.95 (3.43) Aa	4.35 (3.50) Ba
Middle	8.05 (4.05) Aa	4.79 (2.15) Bab
Apical	9.29 (3.99) Aa	7.60 (3.83) Ab

[a] *Matching capital letters refer to equality in the same line (Mann-Whitney Test, p≤0.05). Matching lowercase letters refer to equality in the same column (Kruskal-Wallis Test, p≤0.05).*

RESULTS

Table 2 describes the means of the push-out bond strength (MPa) obtained from the experiment. The bond strength was significantly higher in groups C (p=0.028) and M (p=0.016) when U100 was used, whereas it was similar for both cements in group A. The light-curing access showed a significant effect on the bond strength only when ARC/SBMP was used (p=0.031). The bond strengths of posts cemented with ARC/SBMP were significantly higher in group A compared to group C but did not differ between the A and M groups.

In the failure analysis (Figure 3), the ARC/SBMP cement exhibited a predominance of adhesive failures between the cement and the dentin (95%) in groups C and M with one single mixed failure in each group (5%). Group A exhibited a reduction of adhesive failures between the cement and dentin (65%), an increase in mixed failures (20%), and adhesive failures to posts (15%). With regard to cement U100, group C exhibited 50% mixed failures, 45% adhesive failures to dentin, and 5% adhesive failures to posts; group M exhibited 65% mixed failures, 30% adhesive failures to dentin, and 5% adhesive failures to posts; and group A exhibited 50% adhesive failures to dentin, 35% mixed failures, 10% adhesive failures to posts, and one single cohesive failure on resin cement (5%).

DISCUSSION

The aim of the present study was to assess, by means of the push-out test performed 24 hours after cementation, the bond strengths in the apical third of the fiber posts cemented to root canals. The push-out test enables bond strengths to be measured at different sites and accurately represents the bonding

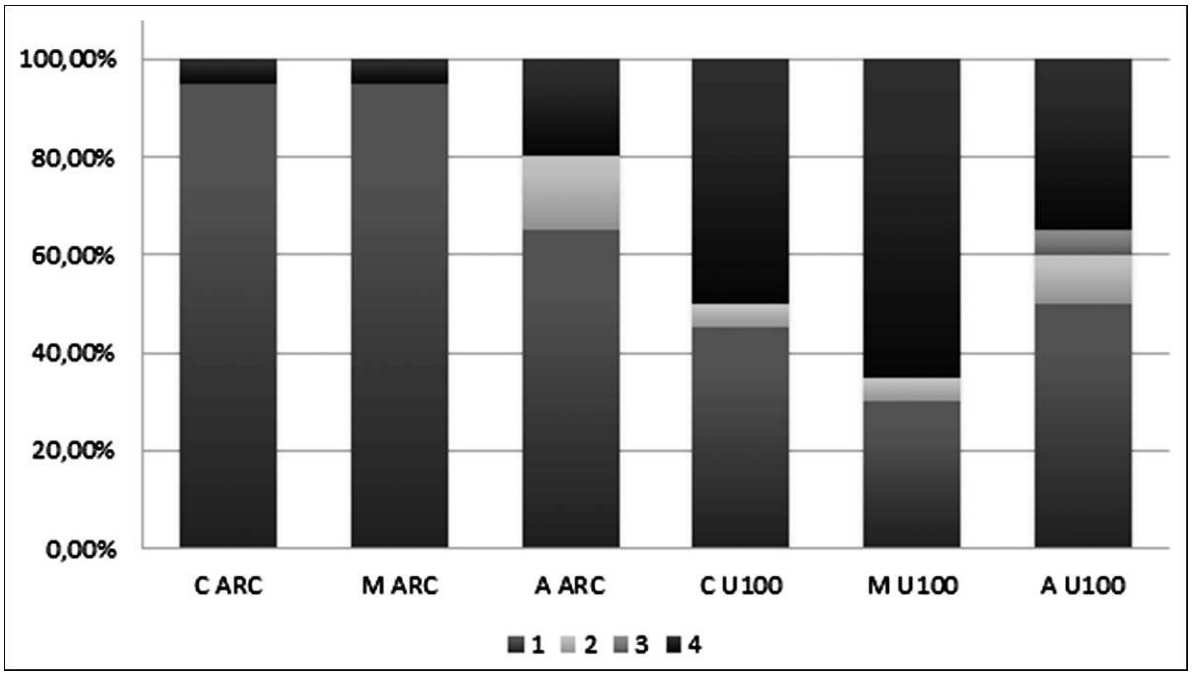

Figure 3. *Types of failures found (n=10). Legend: (1) Adhesive failures between dentin and cement; (2) adhesive failures between posts and cement; (3) cement cohesive failures; and (4) mixed failures.*

conditions of posts in root canals.[17] The photoactivation of resin cements across the entire root canal cannot be achieved in clinical practice. However, the experimental model used herein assumed the hypothetically ideal direct incidence of light on the three root sections to evaluate the effect of light access on bond strength in the apical third of the root. If the approach to light-curing close to the apex became clinically applicable (for instance, in the case of an effective translucent post), the effect of factors other than photoactivation could be further explored.

The null hypothesis of the present study (ie, that neither light access nor cement type influences the bond strengths in the apical third) was rejected. The results showed that ARC/SBMP performed best in the group in which light was directly applied to the apical third and that the U100 cement exhibited better bond strength compared to ARC/SBMP in the groups in which the light was not directly applied to the apical third.

The present study showed that the ARC/SBMP system exhibited lower bond strength in the apical third when the photoactivating light was applied to the coronal level. These results agree with those of previous studies[9-11] showing that dual-cure resin cements depend on photoactivation to achieve the highest values of conversion of monomers into polymers. It was suggested that such lower degrees of conversion result in lower bond strengths at those root-canal depth levels at which photoactivation is ineffective.[17-26,30] In the group in which the light was directly applied to the apical area of the root canal, the bond strength showed significant improvement. Studies have shown that in the apical third, dentin exhibits conditions that are less favorable for adhesion,[14,16] such as a lower number of dentinal tubules,[12-14] and a greater probability of presenting endodontic treatment remnants.[31] However, the results of the present study indicate that the bond strength may be more closely related to the photoactivation condition of RelyX ARC cement. The failure analysis showed that adhesive failures to dentin were predominant in the groups in which the light was not directly applied to the apical third, whereas in the groups in which photoactivation occurred at the apical third, the number of mixed failures increased from 5% to 20% and the occurrence of adhesive failures to posts was 15%. These results indicate that the bonding between the ARC/SBMP system and dentin was more effective in group A, in agreement with the push-out test results, which were higher in that group.

Resin cement U100 exhibited statistically similar values under all conditions investigated, in agreement with previous studies[17,25,26,32,33] that found uniform bond strength values at different root canal levels when U100 was used. In addition, compared to other types of dual cements, the U100 cement appears to polymerize more effectively at different root canal levels.[11] Compared to other self- and dual-cure cements, higher microhardness values were observed at different root canal levels when the U100 cement was used.[11] The performance of U100 at different root canal levels was similar to that of self-activated resin cements, indicating that U100 is likely less dependent on light to attain higher bond strength.[26] The lower variation exhibited by the failure patterns of the various U100 groups provides further evidence of its greater uniformity of adhesion to dentin independent of the site at which light is applied.

The high standard deviation observed in some groups, especially for ARC/SBMP, would be partially explained by the uncontrolled experimental effects such as the variations in the structure of root canal dentin, remnants of root canal obturation materials, moisture control inside the root canal, and technique sensitivity of adhesive application.

Conventional resin cements, such as ARC/SBMP, are based on total acid-etching or self-etching adhesive systems that are associated with low-viscosity resin composites. This multistep technique is complex and highly sensitive and may affect bond quality.[34] Conversely, U100 resin cement involves a simplified technique in which pretreatments of teeth and posts are not necessary.[15] In addition, U100 contains acidic monomers that demineralize and infiltrate the dental structure, thereby promoting micromechanical bonding, and a subsequent chemical reaction promotes integration with hydroxyapatite.[29,31,35] The results of the present study may reflect the effectiveness of that bonding mechanism inside the root canal.

CONCLUSIONS

The type of cement used and the light-curing access level influenced the bond strength between glass fiber posts and root canals. The bond strength of the RelyX ARC/SBMP cement proved to be more dependent on photoactivation than that of the RelyX U100 cement. The light-curing access level did not influence the apical bond strength of RelyX U100. This cement was shown to be suitable for cementing the fiber glass post evaluated in this study.

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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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