OPERATIVE DENTISTRY

March/April 2019
Volume 44
Number 2
109–220

Academy of Operative Dentistry • American Academy of Gold Foil Operators • Academy of R.V. Tucker Study Clubs

OPERATIVE DENTISTRY

Volume 44 / Number 2
March/April 2019

www.jopdent.org

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Clinical Technique/Case Report

Restoration of a Mandibular Incisor With a Computer-aided Design/ Computer-aided Manufacturing Fabricated Anterior Onlay

J May • J Watson

Clinical Relevance

The author proposes a new treatment option for nonsurgical root canal treated mandibular anterior teeth using a computer-aided design/computer-aided manufacturing system without conventional post and core.

SUMMARY

The use of computer-aided design/computer-aided manufacturing (CAD/CAM) dentistry has triggered novel approaches to restoring teeth in ways that increase efficiency, improve esthetics, and conserve tooth structure. Endodontically treated mandibular incisors offer a challenging restorative opportunity due to the small amount of natural tooth structure and the required amount of reduction needed for restorative material. The case presented demonstrates how to leverage chairside CAD/CAM technology to restore a discolored and fractured endodontically treated mandibular incisor without the use of a post and core and completing the restoration in a single visit.

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DOI: http://doi.org/10.2341/18-217-S

INTRODUCTION

Digital and adhesive dentistry procedures have provided conservative alternatives to full coverage crown restoration, which can lead to unnecessary tooth reduction.[1,2] Due to the relative new nature of our modern computer-aided design/computer-aided manufacturing (CAD/CAM) fabricated adhesively applied glass ceramics, there are still discoveries to be made on how to apply these advancements.

Similar to the posterior onlay, the anterior onlay is a concept used to restore esthetic situations where a veneer is no longer the best option but a crown may be unnecessarily destructive.[4] This restorative strategy does not rely on purely adhesive bonding as a veneer does, nor does it rely entirely on traditional mechanical resistance and retention form. A full coverage crown can be bonded, but traditionally significant amounts of dependable bonding substrate, enamel, is removed during tooth preparation, and long-term bonding to dentin has not been shown to be dependable.[5,6] The proposed anterior onlay concept tries to use both ideas to maximize the potential for long-term retention of a restoration. This is accomplished by trying to conserve enamel wherever possible, and additionally trying to maxi-

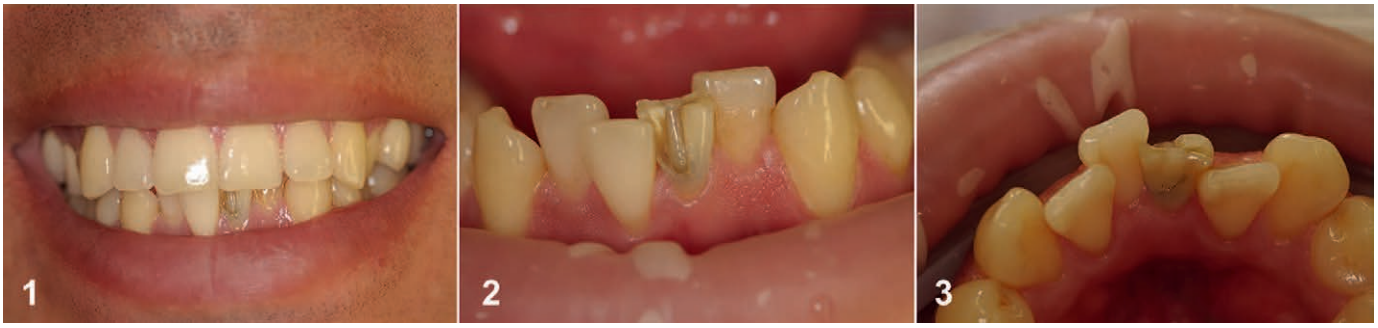

Figure 1. *Initial smile.*

Figure 2. *Existing composite resin core and facially fractured tooth structure.*

Figure 3. *Leaking composite and endodontic access from lingual; the craze line is evident at the distal facial line angle that fractured upon initiation of preparation.*

mize any surface that can provide mechanical resistance and retention.

In the authors' opinion, this restoration design tends to be more ideal in situations where incisal coverage is needed or desired. Whereas a full crown preparation leads to a situation where remaining sound tooth structure is removed and artificial core restorative materials are heavily relied upon to retain the crown. We know that remaining natural tooth structure, and the ferrule effect it provides, is strongly related to the potential longevity of a crown restoration.[7] In addition, the anterior onlay often does not remove more than a minimal amount of tooth structure. Frequently, it can capitalize on the retentive pulp chamber provided after nonsurgical root canal therapy (NSRCT) has been performed, similar to an endocrown where the pulp chamber retained core and crown are a single monolithic restoration.

Fabricating a high-strength glass ceramic restoration using a chairside designed and milled system can be highly efficient, fast, and accurate. In a single one- to two-hour visit, a definitive, highly esthetic, and conservative restoration can be provided to the patient. Additionally, the dentist can maintain control of all aspects of esthetics by controlling the shape and custom staining.

CASE STUDY

A 32-year-old healthy male patient presented with a nonsymptomatic, coronal facial fracture and previous NSRCT of No. 24 (Figures 1 through 3). Due to mandibular anterior crowding, the tooth was not in occlusion. The reason for the previous restoration was trauma when the patient was an adolescent. The patient's chief concern was the unesthetic dark appearance of his tooth.

Mandibular anterior teeth can be difficult to treat due to their relatively small size. In the case presented here, the size of the fractured fragment and need for cuspal coverage made the use of a direct bonded composite resin less than ideal. Often a post and core are necessary to retain a crown after a minimum 1-mm recommended shoulder for most all-ceramic restorations.[8] Nearly all of the remaining tooth structure would have to be included in the preparation. An alternative outcome is to underprepare the tooth, but this leads to a restoration that is overcontoured to maintain minimal thickness for the glass ceramic strength. This results in an unesthetic appearance, and an overcontoured restoration can lead to plaque retention and periodontal disease.[9]

The patient chose the minimally invasive CAD/CAM anterior onlay, which would preserve all tooth structure except for incisal reduction. No anesthesia was necessary because the tooth was already nonvital and all margins would remain supragingival. Isolation was obtained (Optragate, Ivoclar Vivadent, Amherst, NY, USA; Isodry, Isolite Systems, Santa Barbara, CA, USA). The remaining composite was removed. Staining extended 1 mm into the pulp chamber. A fracture line was seen at the distal line angle of the facial surface (Figure 3). When initiating tooth preparation (fine diamond, flat-end taper, Neodiamond Microcopy Kennesaw, GA, USA), the distofacial fragment fractured. The final onlay preparation margin was determined by the fracture on the facial and the endodontic access on the lingual. The interproximal finish lines were the result of the incisal 2-mm reduction. All margins were smoothed with the same fine diamond, and internal corners were rounded. A shade A1 glass ionomer (GC Fuji IX GP, GC Corporation, Tokyo, Japan) orifice barrier

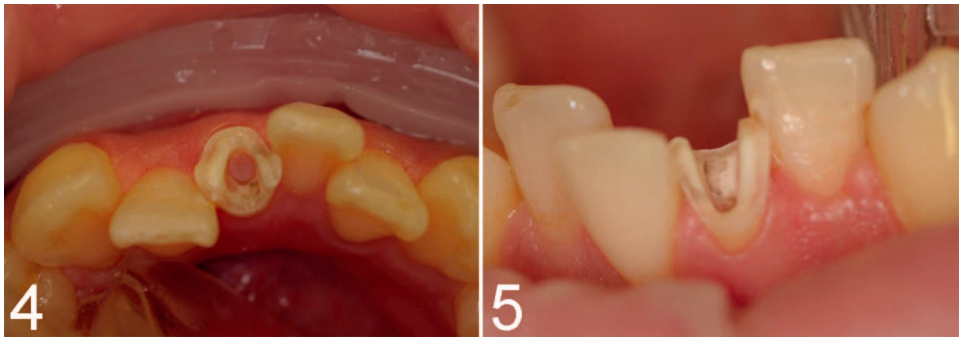

Figure 4. *Glass ionomer core placed, and final preparation showing pulp chamber and lateral wings for retention, and 360° of enamel for resin bonding.*

Figure 5. *Preparation from the facial view.*

was placed to seal the NSRCT-treated tooth, block out undercuts, and flatten the floor of the pulp chamber (Figure 4). Rather than fill the whole pulp chamber with a core, we elected to use the pulp chamber as an endocrown to increase the mechanical resistance and retention without having to remove any additional tooth structure (Figure 5). Interproximal contacts were not broken, and since the process would be all digital rather than analog, there was no need to separate the teeth with a finishing strip as we would for traditionally prepared veneers.

Preparation design was very conservative. The only tooth structure removed was the incisal reduction, much of which was already compromised. Resistance and retention form were maximized with the remaining 2-mm-high proximal tooth structure conserving natural contacts. This, in combination with the 3-mm internal retention gained from the pulp chamber, resulted in a 5-mm depth of resistance and retention form. In addition, the preparation had 360° of substantial enamel remaining along all of the margins. Being supragingival there was no need for gingival retraction.

An impression was digitally captured (CEREC Bluecam, Sirona, Charlotte, NC, USA), and a proposal was created with CAD software. A lithium disilicate block (A1-LT-12) was selected (IPS e.max CAD, Ivoclar Vivadent, Amherst, NY, USA), and the restoration was milled (Cerec MCXL mill, Sirona). The precrystallized restoration was tried in intraorally so any adjustments could be made easily at this stage before crystallization and custom staining was done. Although the tooth was not in function, the author (JM) elected to polish the incisal 1 mm of the restoration (Dialite LD, Brasseler USA, Savannah, GA, USA), as glaze can wear away in function, and when it does, it is essential that occlusal contacts only touch polished porcelain/ceramic to avoid excessive wear.[10] The restoration was then custom stained, glazed (IPS Empress Universal Shade/Stains, Ivoclar Vivadent), and crystallized in a ceramic oven.

The final restoration was then prepared for bonding following manufacturer's recommendations for lithium disilicate using 5% hydrofluoric acid for 20 seconds (IPS Ceramic Etching Gel, Ivoclar Vivadent) and silane (Monobond Plus, Ivoclar Vivadent). The tooth was prepared for resin cement by selective enamel etching for 15 seconds and resin bonding agent application (Total Etch 37% phosphoric acid, Adhese Universal resin bonding agent, Variolink II resin cement, Ivoclar Vivadent). The dual-cure resin cement was syringed into the tooth pulp chamber and then onto the intaglio surface of the restoration and delivered. Excess cement was removed, and the restoration was light cured (ESPE Elipar S10, 3M, St Paul, MN, USA) following manufacturer's instructions. Margins were polished and final occlusion and interproximal contacts checked (Figures 6 and 7). There was no need for

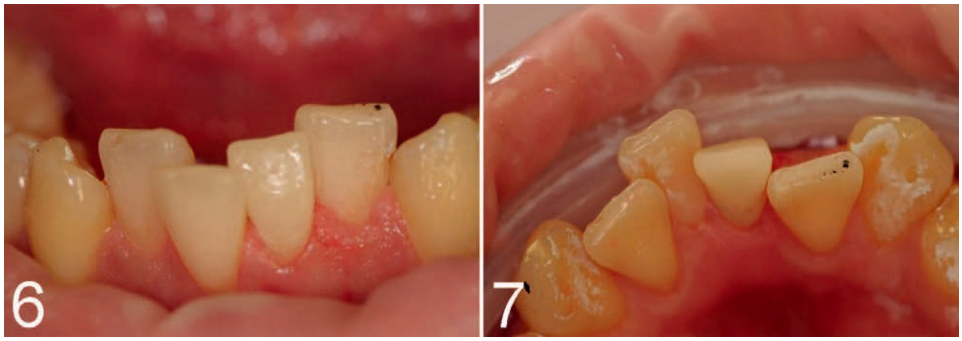

Figure 6. *Final custom-stained lithium disilicate restoration delivered and margins polished.*

Figure 7. *Final restoration from the occlusal view.*

anesthesia or retraction cord; in addition, the simplicity of the preparation and the use of limited restorative materials led to a total treatment time under two hours.

DISCUSSION

Sometimes restorative needs move past reliable use of a direct restorative material due to quality and quantity of remaining enamel and dentin.[11] In posterior teeth, typically when cuspal coverage is required, the next minimally invasive restorative options are onlays. It is not until damage is severe that the conservative recommendation becomes a full crown. Historically, anterior teeth have not had a similar intermediate restorative option between veneers and full-coverage crowns. With the ability to create chairside restorations using CAD/CAM systems, the authors suggest an anterior onlay concept in an effort to conserve tooth structure before removing most of the tooth required for full crown preparation. This can be especially advantageous after endodontic therapy and when more dentin is exposed than is ideal for laminate veneers. These may be especially useful when enamel is still found 360° around the preparation, and mechanical resistance and retention can still be incorporated into the preparation without removing additional tooth structure. When onlays fail, crowns can often still be restorative options. Conversely, when a crown fails, another crown may not be possible.

When laminate veneers are prepared that do not fully break, interproximal contact finishing strips are used to very lightly open the contact. This allows separation of the die stone without having to risk snapping or breaking the stone after sectioning. With digital technology this is no longer necessary because instead of stone a digital model is used, which cannot fracture. However, when a margin ends in an interproximal contact, it can be difficult to prepare a margin at this location and clearly capture the margin in the digital impression. The authors have found that a small amount of local anesthetic infiltration just below the gingival papilla allows the placement of a wedge for separation. This can aid the safe placement of the margin preparation with less risk of damage to the adjacent tooth, and the slight amount of separation makes it easier to capture the margin in a digital impression. In the authors' experience. pre-wedge placement does not affect the software's ability to create a proposal for the restoration. To account for the separation of the wedge, the interproximal contact on that side of the proposed restoration can be lightened. After milling, the restoration can be tried in and adjusted before being bonded into place with a resin luting cement.

Another concern is the ability of the software to understand what is desired from this type of unconventional preparation. The authors' experience has shown that the software has no difficulty proposing appropriate restorations to accommodate anterior onlay preparations. The chairside CAD software readily proposed a clinically desired restoration and only requires minimal operator design modifications.

An endocrown is a monolithic pulp chamber retained core and crown restoration. Research has generally shown that endocrowns seem to have similar, if not better, survival rates in comparison to a composite core or post and core systems under a crown.[12] Information gained from the testing of novel restorative designs, such as endocrowns, are what the authors use to justify this preparation.[12] This restoration design can be more efficient than completing an additional core placement procedure.

CONCLUSION

Anterior onlays avoid unnecessary tooth reduction compared with full crown preparations. The anterior onlay concept offers an intermediate restorative option in cases of anterior teeth that need incisal coverage but lack the ideal parameters for a laminate veneer. This is especially advantageous when restoring an endodontically treated tooth where the pulp chamber can offer additional retention. This technique allows preparations to be guided by remaining tooth structure and previous existing restorations to maximize utilization of existing internal and external retentive features. The use of modern in-office CAD/CAM systems with this type of restoration can lead to a very esthetic outcome that results in a minimally invasive treatment in a single appointment.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Indiana University.

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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.

(Accepted 20 September 2018)

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Clinical Research

A Randomized Double-blind Clinical Trial of Dentin Surface Treatments for Composite Restorations in Noncarious Cervical Lesions: A 36-month Evaluation

AC Rocha • MM Suca Salas • AS Masotti • WLO da Rosa • CH Zanchi • RG Lund

Clinical Relevance

Treating dentin surfaces with an ultrasound probe provides similar clinical performance to conventional techniques while increasing acid-etching time results in a higher risk of failure over time.

SUMMARY

Objective: This randomized, double-blind clinical trial aimed to evaluate the influence of different dentin surface treatments in noncarious cervical lesions (NCCLs).

Methods and Materials: Twenty-nine patients participated in this study. One hundred sixty-five NCCLs were selected and randomly assigned to three groups: G0 (control group) with phosphoric acid etching for 15 seconds; G1: phosphoric acid etching for 30 seconds; and G2: ultrasound probe applied for 30 seconds on the dentin surface. Class V composite resin restorations were performed (Z350, 3M ESPE, St Paul, MN, USA). The restorations were evaluated at baseline and at six, 12, 24, and 36 months according to the World Dental Federation criteria. Survival curves were obtained using the Kaplan-Meier method and the log-rank test. Comparisons between groups and times were performed using the McNemar and Chi-square tests (α=0.05).

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DOI: http://doi.org/10.2341/17-308-C

Results: The presence of failures due to retention was statistically different among the groups (p=0.012), and G0 and G2 showed better clinical performance than did G1. Sensitivity decreased over time in all groups. Marginal discoloration, postoperative sensitivity, and marginal adaptation were not different among the groups (p>0.05).

Conclusions: The studied dentin surface treatments showed similar clinical performance to the conventional technique at 36 months in terms of marginal discoloration, marginal adaptation, and postoperative sensitivity. In contrast, increased acid-etching time resulted in a higher risk of failure due to retention over time in composite Class V restorations.

INTRODUCTION

Noncarious cervical lesions (NCCLs) are frequently found in adults during clinical practice.[1,2] Almost a quarter of the world's population presents NCCLs, mainly in the premolars.[3] The prevalence of NCCLs has been associated with aging and tooth wear. Often, these lesions need to be restored because of sensitivity, esthetic reasons, or even to prevent further progressive loss of dental structure and to decrease plaque retention.[4,5] Treating NCCLs is often complicated because the operative field must be dry during the restorative procedure, which is clinically difficult because of the proximity of the lesion to the gingiva.[2]

In NCCLs, the critical point of adhesion is the union between the dental structure and the restorative material, especially when a cavity has poor or no retentive features.[6] Thus, to preserve the tooth structure, no dentin surface treatment is performed, or, on the contrary, numerous treatments are adopted that could vary according to the size of the cavity, the dentin substrate, and the type of restorative material to be used.[7] Studies[8,9] have investigated the effect of different dentin surface treatments on the microleakage and adhesion of composite resins in permanent teeth. A drawback of the use of composites in NCCLs might be the substantial differences in the composition of the bonding surface, since NCCLs are mainly located in the dentin, wherein bonding is more difficult to achieve than in the enamel.[9,10] NCCLs also have a high degree of sclerosis, which makes the formation of the hybrid layer on such hypermineralized dentin more difficult.[11,12] In this context, mechanical and chemical dentin surface treatments have been suggested in Class V composite restorations.[5,8-10,13-17]

Dentin surface treatments include the removal of sclerotic dentin, selective enamel etching with phosphoric acid, dentin roughening with a diamond bur, and preliminary etching with ethylenediaminetetraacetic acid.[18-21] Some clinical studies[5] have shown that these surface treatments can improve the retention rate of composites in NCCLs. Other alternative dentin treatments that could be beneficial in Class V restorations are longer durations of acid-etching application and the use of ultrasound on the dentin surface. Only *in vitro* studies have evaluated different application times of acid etching prior to composite restoration in NCCLs,[22-24] and these studies have shown contrasting results. No clinical trials have evaluated the use of ultrasound in NCCLs. Dentin surface treatments aim to increase the longevity of restorations, which is important to prevent the continued restorative cycle and preserve the dental structure and pulp vitality. Therefore, the aim of this randomized, double-blind clinical trial was to evaluate the influence of acid etching and ultrasound dentin surface treatments on the survival of composite Class V restorations. The hypothesis evaluated was that dentin surface treatments would improve the following outcomes: fracture and retention, marginal adaptation, marginal discoloration, and postoperative sensitivity.

METHODS AND MATERIALS

Sample Size Calculation

The sample size was calculated using an average annual failure rate of 6% for composite Class V restorations performed with conventional adhesive systems,[25] with 80% power and a significance level of 5%. The final sample was 28 patients to be monitored for a period of five years, considering a possible loss of 20%.

Patient Selection

The selection of patients was conducted through the dissemination of posters and distribution of pamphlets with information regarding the study in the School of Dentistry, Federal University of Pelotas, Brazil, and in basic health units located in the urban area of Pelotas. This study was approved by the local ethics committee (IRB approval process 035-2011). Patients previously diagnosed with NCCLs were scheduled for a new clinical examination to evaluate if they could be included in this study according to

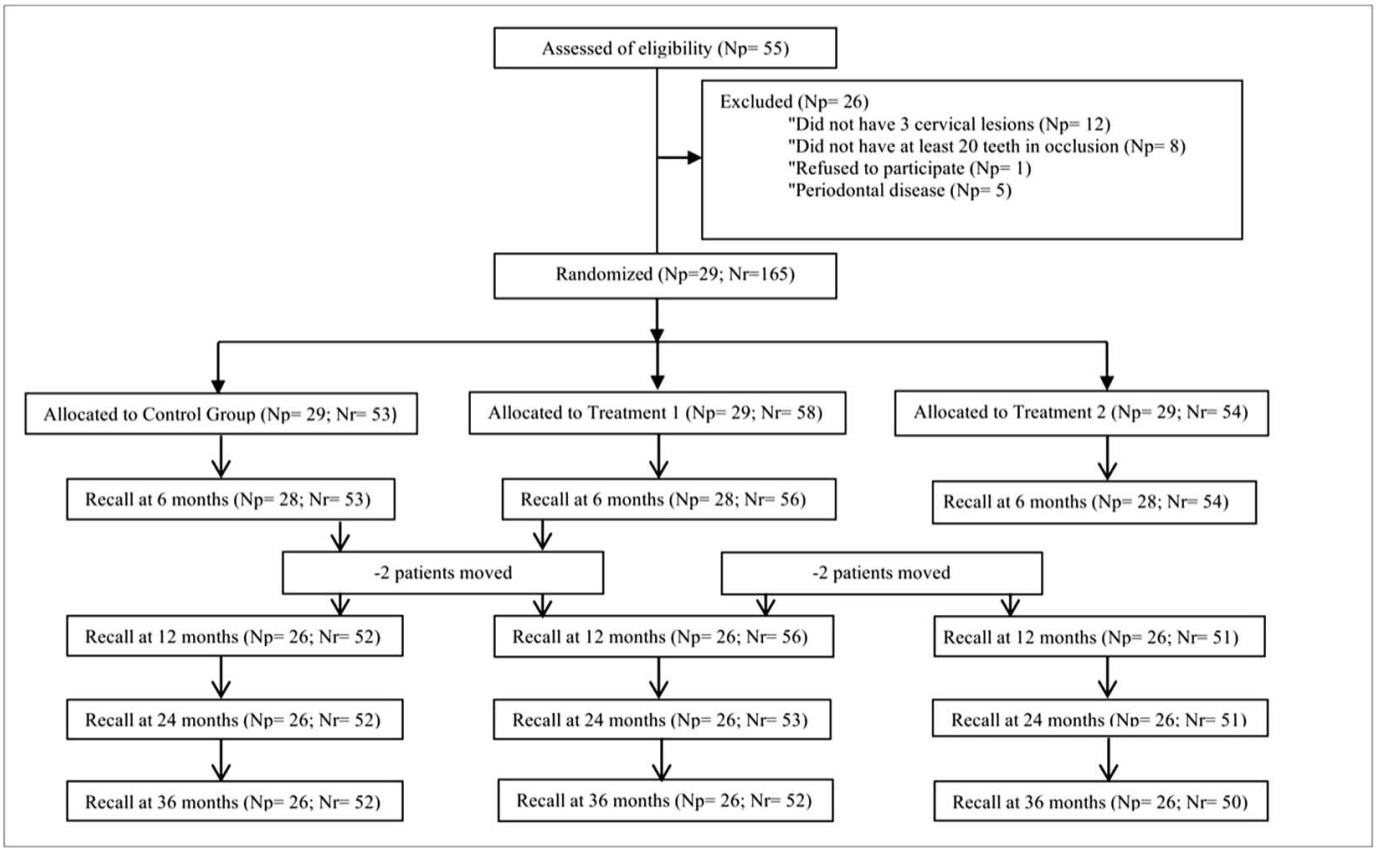

Figure 1. *Flow diagram illustrating the study stages. Np = number of patients; Nr = number of restorations.*

the inclusion and exclusion criteria. Written informed consent was obtained from all patients prior to starting the treatment. Based on preestablished criteria, we selected 29 patients for this study (Figure 1).

Eligibility Criteria

The included patients had to present at least three NCCLs on the buccal surface of vital anterior or premolar teeth. In addition, they had to present with less than 20% of visible plaque index and/or gingival bleeding index, NCCLs with no more than 3-mm probing depth, and good general health. Patients who presented with less than 20 teeth in the oral cavity; those undergoing orthodontic treatment; those with occlusion problems, with caries lesions or restorations on the buccal surface of a tooth, or with veneers or crowns; and those without antagonist teeth were excluded. Patients who met the eligibility criteria received an information letter and an informed consent form to be signed.

Training and Calibration Process

Six dentistry students enrolled in the seventh and eighth semesters participated in the training process. In the first stage of training, a two-hour lecture regarding the materials and techniques used for the removal or modification of the hypermineralized surface layer of non-carious cervical lesions was conducted. A routine detailed protocol to be instituted during the sessions was also presented. A manual containing the instructions and the protocol of clinical procedures was created and given to the students.

In the second stage of training, clinical activities regarding composite Class V restorations were performed in mannequins and in volunteers presenting NCCLs with restorative needs. These volunteers were not included in the study.

At the end of training and calibration, two operators were selected from among the six students initially enrolled to perform the restorative procedures. In addition, two assistants supported the operators and completed the medical records. The

other students were in charge of sterilization of the instruments, scheduling appointments, molding, creating photographic records, oral hygiene orientation, and periodontal treatment; all of these were among the other dental procedures offered to the patients. All of the above-mentioned steps were performed under the direct supervision of a researcher with experience in the field.

Clinical Examination

Clinical assessments were performed in the dental clinic of the Faculty of Dentistry of the Federal University of Pelotas by two previously calibrated examiners, under the supervision of a researcher responsible for the study.

The presence of NCCLs was assessed by visual inspection using a dental mirror. After confirming the presence of NCCLs, the patient's medical record containing identification data and general and dental history was completed. Dental examinations were performed using a dental mirror, a periodontal probe, dental tweezers, cotton rolls, and a dental aspirator.

For the calibration step, the study director placed one restoration within each group in order to identify all the steps involved in the application technique. Thereafter, the operators placed three restorations in 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.

The randomization process in selecting the dentin surface treatment for each NCCL in the patients was performed using identical opaque envelopes and simple selection by the blinded operators. Allocation assignment was revealed by opening the envelope at the time of the restorative procedure.

Preparation of the Patients

Four weeks before the start of the study, the patients were subjected to a prophylaxis session, which involved smoothing and polishing the supragingival tooth structure. Patients received individualized instruction for the mechanical control of dental biofilm, including guidance on brushing technique and flossing. During the monitoring period, dental support was also offered to patients involved in the study.

Clinical Protocol

Prophylaxis was conducted using a glass and rubber dam–based slurry of pumice and water. The color of the composite was selected using a shade guide (Vitapan Classical, Vita Zahnfabrik, Bad Säckingen, Germany). Local anesthesia was used if necessary before the restorative procedure.

Isolation was accomplished using a lip retractor, retraction cord #000 (Ultrapak Cord, Ultradent, South Jordan, UT, USA), cotton rolls, and a dental aspirator. Cotton rolls were positioned in the upper labial sulcus, in the lower labial sulcus, and in the sublingual region to absorb the saliva flowing mainly from the major salivary glands. The retraction cord was inserted into the gingival sulcus with the aid of a blunt spatula without excessive pressure to the periodontium. Preparation or beveling of cavosurface margins was not performed.

Teeth were randomly divided into three groups according to the techniques used to treat the hypermineralized ultra–surface layer dentin of the NCCLs, as follows:

- Control group (G0): 15-second dentin etching with phosphoric acid (protocol recommended by the manufacturer);
- Experimental group 1 (G1): 30-second etching performed with 37% phosphoric acid gel, prior to the application of the resin adhesive; and
- Experimental group 2 (G2): Ultrasound (EMS Mini Piezon) probe (Instrument B part°: DS-003A) applied for 30 seconds (vibration range from 25,000 to 32,000 pulses/s) on the hypermineralized NCCL dentin surface.

Restorative procedures were performed using a conventional adhesive system (Single Bond II, 3M ESPE, St Paul, MN, USA) and restorative nanoparticulate composite (Filtek Z350, 3M ESPE), following the manufacturer's instructions. The restorations were placed using an incremental technique with about two or three increments of the restorative composite according to the size of the NCCL using spatulas, brushes, and silicone tips for the composite resin. An LED device (Radii Cal, SDI, Victoria, Australia) with an intensity of 1200 mW/cm^2 was used for polymerization. The restoration was finished using a #12 scalpel blade and diamond and fine-grained multilaminated drills in order to remove excess material and/or improve the shape of the contour restorations. Polishing was done with silicone tips, flexible abrasive discs (Sof-Lex Pop-On, 3M ESPE), felts, and polishing discs (3M ESPE).

Clinical Evaluation

Assessment of the restorations was performed by two examiners (graduates in the field of dentistry and

Table 1: *World Dental Federation (FDI) Criteria Used for Clinical Evaluation*[49,50]

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

different from the two operators), previously trained and calibrated with at least 80% intra- and inter-examiner kappa. In the event of a disagreement, direct clinical reevaluation of restorations or evaluation of digital photographs using the clinical criteria was performed, and discussion was conducted to reach a consensus. The examiners were blinded to the experimental groups and clinical assessment was made independently using magnification, mirror, explorer, millimeter periodontal probe, clinical tweezers, cotton rolls, and a dental aspirator. Pulp vitality was measured using cotton balls imbibed with gas jet cooling at −50°C on the surface of the teeth. Dentin sensitivity was evaluated after air jet application for three seconds at a distance of 2-3 cm from the buccal surface. Results were dichotomized as "Yes" or "No." The evaluators also referred to the digital photographs during evaluation.

Factors evaluated included pain perception sensitivity, color change and integrity of the restoration. Clinical evaluations were performed at one week (baseline) and six, 12, 24, and 36 months after the insertion of the restorations using the World Dental Federation (FDI) criteria (Table 1). Loss of restorations was considered a failure due to retention issues.

Statistical Analysis

The statistical analyses followed the intention-to-treat protocol according to the CONSORT suggestion.[26] This protocol includes all participants in their originally randomized groups, even those who were unable to adhere to their scheduled follow-up visits. This approach is more conservative and less open to bias. Data were tabulated, and statistical analyses were performed using the Stata 12.0 software package (Stata Corp LP, College Station, TX, USA). To report the frequency distribution of the evaluated criteria, descriptive statistics were used.

Table 2: *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.*[27]

Survival curves were obtained using the Kaplan-Meier method and log-rank test for comparison between groups. Comparisons between groups and between study periods were performed using the McNemar test and Chi-square test, respectively, considering a significance level of 5%.

RESULTS

Descriptive Analysis

Restorations were placed between March 2011 and February 2013. The follow-ups started in July 2012 and ended in February 2016. The control and two experimental groups were placed exactly as planned without modification. One patient did not attend the six-month follow-up, and two patients did not attend the 12- and 36-month follow-ups (Figure 1). The reasons for follow-up losses were relocation from the city and loss of contact information (ie, telephone number or address).

The distribution of NCCLs in the 165 teeth of the 29 patients was almost equivalent between the maxillary and mandibular dentition. The distribution was 13.9% and 15.2% in the incisors and canines, respectively. The NCCLs were mainly localized in the premolars (ie, in more than 70% of the cases). In addition, the shape of the lesions was mostly circular (69.2%), with a diameter (64.8%) and depth of up to 2 mm (64.2%). Clinical observations based on color (Table 2)[27] showed that sclerosis was absent in more than 70% of the lesions, and the degree of sclerosis was mild or moderate in more than 28% of the lesions. Preoperative sensitivity was absent or light in more than 60% of the teeth and moderate-severe or severe in an average of 30% of teeth.

Of the 29 patients, 18 were women and 11 were men. The predominant age was between 49 and 58 years old, accounting for 42.9% of the patients, and over 82% of the patients were 37 years or older (Table 3). The main reason for restoration indicated by the patients was the limitation of lesion progression, corresponding to more than 64% of the responses, followed by esthetic dissatisfaction (42.9%) and dentin hypersensitivity (39.3%). The sample was also categorized according to smoking, daily intake of acidic beverages/foods, presence and types of harmful habits, classification of occlusion, and lateral guidance.

Table 4 shows the two experimental treatment groups and the control group at different times of evaluation of the restorations. In the control group (G0), only one failure (loss of restoration) was observed at the 36-month evaluation; G1 had a greater number of failures of restorations, at seven failures. In G2, two failures were observed at 24 months and two at 36 months. At the end of 36 months, 12 restorations lost in the three treatment groups could be aggregated.

Retention

Failure incidence increased and was statistically different at baseline and at 12, 24, and 36 months ($p<0.05$). The differences were also observed between six months and 24 and 36 months of evaluation ($p<0.05$). Loss of restorations after 36 months were more common in the mandibular (63.6%) premolars (72.7%), with lesions presenting shape angles of more than 135° (72.7%), 3- to 4-mm height (36.4%), and 1- to 2-mm depth (72.7%). No significant differences were observed between baseline and six months, between six months and 12 months, and between 12 months and 24 and 36 months.

In the control group, failure incidence was similar among the studied periods. In G1, more failures were observed at 24 and 36 months than at baseline ($p<0.05$), and the incidence was increased significantly more between 12 and 24 months than at 36 months ($p<0.05$). In G2, failure incidence increased from baseline to 36 months and between six and 36 months ($p<0.05$).

Figure 2A shows the Kaplan-Meier survival graph for failures of the composite. Failure incidence increased over time and was different among the groups (log-rank: $p=0.0124$).

Table 3: *Distribution of Noncarious Cervical Lesions (NCCLs) According to the Studied Patients (Sex and Age) and the Characteristics of Class V Lesions (Shape, Cervico-incisal Size of the Lesion, Degree of Sclerotic Dentin, Presence of Antagonists, Presence of Attrition Facets, Presence of Preoperative Sensitivity, and Tooth and Arch Distribution)*

Characteristics of Research Subjects	No. of Lesions/ Subjects	%
Gender distribution		
Male	11	37.9
Female	18	61.1
Age distribution, y		
18-29	02	6.9
30-39	07	24.1
40-49	06	20.7
>49	14	48.3
Characteristics of Class V lesions		
Shape, ° of angle		
<45	58	35.2
45-90	—	—
90-135	—	—
>135	107	64.8
Cervico-incisal height, mm		
<1.5	11	6.7
1.5-2.5	106	64.2
2.5-4.0	39	23.6
>4.0	09	5.5
Degree of sclerotic dentin		
1	111	67.3
2	39	23.6
3	15	9.1
4	—	—
Presence of antagonist		
Yes	152	92.1
No	13	7.9
Attrition facet		
Yes	—	—
No	29	100
Preoperative sensitivity (spontaneous)		
Yes	128	77.6
No	37	22.4
Preoperative sensitivity (air dry)		
Yes	110	66.7
No	55	33.3
Tooth distribution		
Anterior		
Incisor	23	13.9
Canine	25	15.2
Posterior		
Premolar	117	70.9
Arch distribution		
Maxillary	80	48.5
Mandibular	85	51.5

Marginal Staining /Marginal Discoloration

Marginal discoloration increased significantly ($p<0.001$) from baseline to all the other evaluation times (six, 12, 24, and 36 months). Marginal discoloration after 36 months was more common in the maxillary (52.5%) premolars (67.5%), with lesions presenting 1- to 2-mm height (55.3%) and depth (76.3%). In G0, G1, and G2, baseline staining increased at six, 12, 24, and 36 months ($p<0.001$). In G2, marginal discoloration increased between baseline and 36 months and between six and 36 months ($p<0.05$).

Marginal discoloration observed over the studied periods showed no differences among the groups (log-rank: $p=0.8588$) (Figure 2B). At 36 months, G2 presented more marginal discoloration than did G1 and G0, albeit with no statistical differences.

Marginal Adaptation

Marginal adaptation was not significantly different among the groups (log-rank: $p=0.1744$) (Figure 2C). The Kaplan-Meier survival graph showed that G1 maintained better marginal adaptation than did G0 and G2.

Postoperative Sensitivity

Dentin sensitivity decreased significantly between baseline and the four evaluation times: six, 12, 24, and 36 months ($p<0.001$). Comparisons between six and 36 months showed an increase in sensitivity ($p<0.001$). A similar situation occurred between 12 and 36 months and between 24 and 36 months, wherein sensitivity increased significantly ($p<0.001$). Sensitivity after 36 months was more common in the mandibular (54.5%) premolars (68.8%), with lesions

Table 4: *Number of Restorations in Each Experimental Group Classified According to the World Dental Federation (FDI) Criteria*[49,50]

Hickel Criteria[a]	Baseline			6 mo			12 mo			24 mo			36 mo		
	G0	G1	G2	G0	G1	G2	G0	G1	G2	G0	G1	G2	G0	G1	G2
1. Marginal staining															
VG	53	58	54	53	57	53	52	54	48	51	52	45	50	50	44
GO	—	—	—	—	01	01	01	04	06	02	06	09	03	08	10
SS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
UN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2. Fractures and retention															
VG	53	58	54	53	56	54	53	57	50	52	54	50	52	55	50
GO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SS	—	—	—	—	—	—	—	—	01	—	01	02	—	02	02
UN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PO	—	—	—	—	02	—	—	01	—	—	03	02	01	01	02
3. Marginal adaptation															
VG	53	58	53	53	58	52	53	57	54	53	57	53	52	58	52
GO	—	—	—	—	—	02	—	01	—	—	01	01	01	—	02
SS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
UN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4. Postoperative sensitivity															
VG	53	58	54	50	57	53	51	56	51	52	58	53	51	56	52
GO	—	—	—	03	01	01	02	02	03	01	—	01	02	02	02
SS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
UN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5. Secondary caries															
VG	53	58	54	53	58	54	53	58	54	53	58	54	53	58	54
GO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
UN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations: G0, control group; G1, increase the etching time; G2, ultrasound probe.
[a] *VG, clinically very good; GO, clinically good; SS, clinically sufficient/satisfactory; UN, clinically unsatisfactory; PO, poor.*

presenting shape angles of more than 135° (55.0%) and 1- to 2-mm height (55.0%) and depth (70.0%).

In G0, G1, and G2, baseline sensitivity decreased significantly at six, 12, 24, and 36 months ($p<0.001$). In G0, sensitivity was higher at 36 months than at 24 months ($p=0.04$). In G1, sensitivity was significantly higher at 36 months than at six, 12, and 24 months ($p<0.001$). In G2 as well, sensitivity was significantly higher at 36 months than at six, 12, and 24 months ($p<0.05$).

Dentin sensitivity was not different among the studied groups (log-rank: $p=0.4941$) (Figure 2D). The Kaplan-Meier survival graph suggested that sensitivity was more common in G0 than in G2 and G1. G1 had slightly fewer cases of sensitivity.

Other Parameters

No restoration had clinical problems related to the recurrence of caries at 36 months according to the FDI criteria. In addition, no adverse effects were reported by any patient regarding the treatments performed.

DISCUSSION

Previous studies[28] reported that Class V composite restorations in NCCLs have limited clinical durability. The most frequently cited reasons for possible

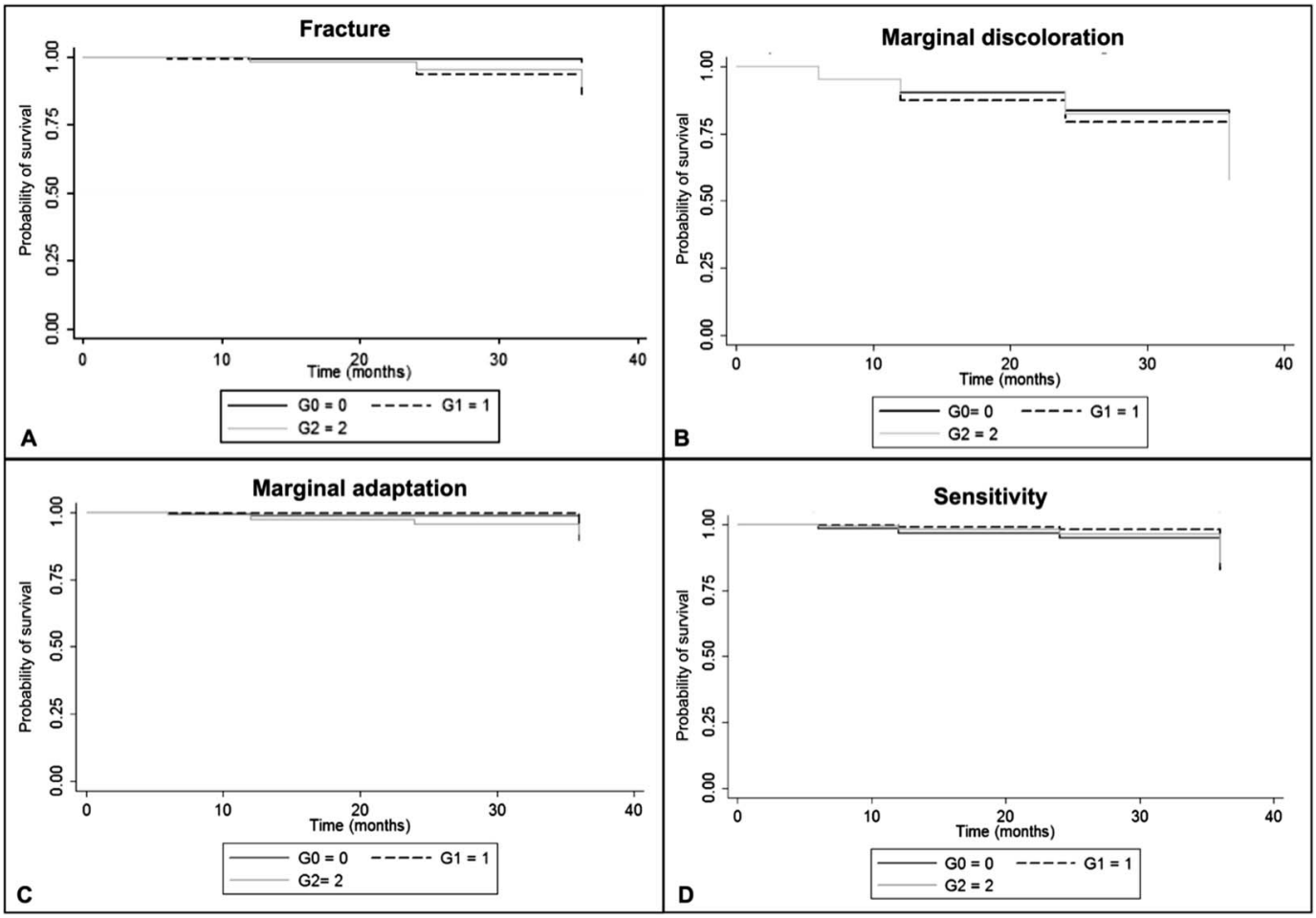

Figure 2. *Kaplan-Meier survival curves. (A) Clinical performance (failure) according to the experimental groups (log rank:* p*=0.0124). (B) Marginal discoloration according to experimental groups (log rank:* p*=0.8588). (C) Marginal adaptation according to experimental groups (log rank:* p*=0.1744). (D) Sensitivity according to experimental groups (log rank:* p*=0.4941).*

failures, even within a short period of time, included the loss of retention and poor marginal adaptation.[25,29] The loss of retention and poor marginal adaptation of the restorations performed in our study showed satisfactory long-term survival, and 12 restorations were lost, possibly because of the failure of adhesion to the substrate. The teeth exposed to a longer acid-etching time as part of the experimental dentin surface treatment showed inferior clinical performance compared with the teeth in other experimental groups. In addition, dentin surface treatment with ultrasound showed similar restoration survival to the control group after the 36-month evaluation period. Considering the aforementioned findings, our hypothesis was rejected, because the tested dentin surface treatments did not improve the survival of Class V composite restorations.

Different strategies were used in previous studies[12,30] to increase the bond strength of adhesive systems to sclerotic dentin, such as bur removal of the most superficial sclerotic dentin layer or pre-etching with phosphoric acid. There is a consensus in the literature that sclerotic dentin exhibits a low-permeability hypermineralized surface, resulting in a substrate that is less favorable for adhesion than is normal dentin,[31,32] which is a substrate usually found in NCCLs.[30] The sclerotic dentin consists of high amounts of mineral precipitates that present increased surface roughness, high surface energy, and less intercollagen infiltration; therefore, bonding to this kind of dentin was compared with bonding to a two-times etched enamel.[16] In the present study, the higher loss rate observed in the experimental group with increased acid etching could be due to the presence of nonsclerotic dentin observed in the majority of teeth. Using the recommended etching times, the thickness of the hybrid layer may change abruptly because of uneven etching.[12] This fact could

be even worse when the phosphoric acid–etching time is duplicated and may account for the controversial results observed when the etching time is duplicated in sclerotic dentin.[1,12,33] Moreover, the duplication of the phosphoric acid–etching time could cause the deepest demineralization of some intertubular and peritubular dentin, which could not be thoroughly infiltrated by resin monomers.[34]

However, the majority of restorations conducted in this study had nonsclerotic dentin, which was classified according to specific criteria for these types of lesions.[27] In these cases, a longer acid-etching time could increase the chances of degradation of collagen fibrils, compromising the bond stability over time.[35-37] Overetching, with subsequent deep demineralization, can lead to suboptimal resin impregnation and to a porous zone in the hybrid layer.[38,39] In the long term, this could compromise the durability of the bond.[34,40,41] If surface demineralization is too deep, there is a chance that the resin monomers will not diffuse to the full depth of the demineralized dentin. This would leave collagen fibers exposed and susceptible to hydrolysis, possibly weakening the bonding. Thus, it is speculated that superficial demineralization may possibly give the adhesive system a better chance to diffuse into the entire collagen network. In this context, a minimum acid-etching time of 15 seconds has been suggested by several authors,[42] with the aim to achieve an adequate bond to the normal dentin. Some *in vitro* studies[43,44] reported that increasing the etching time to 30 seconds apparently does not affect the bonding of the current hydrophilic adhesive systems to normal dentin, at least in terms of bond strength. However, in our clinical trial, a longer acid-etching time affected the retention of Class V composite restorations. In clinical practice, defining the degree and volume of sclerotic dentin is difficult, and the correct diagnosis of sclerotic dentin that will guide the clinical procedure can also be difficult.[16]

In our study, the increased etching time did not influence marginal adaptation more than it did in the other experimental groups. This possibly occurred because of better adhesion to the enamel.[16] The optical time for acid etching in an enamel substrate is 30 seconds, the time period that was used in our clinical trial. However, the presence of enamel in NCCLs and its influence on adhesion are debatable.[23] The marginal sealing ability of a restorative material in dentistry could not necessarily be correlated with caries formation, but the development of microleakage over time can influence the longevity of the restoration for esthetic reasons, especially in the visible areas of the anterior region.[45] One study[9] that evaluated bur removal of dentin and additional phosphoric acid etching before self-etch primer application showed better marginal adaptation of both self-etch adhesive systems but did not change the retention rates of Class V composite restorations.

Our study also analyzed dentin surface treatment with ultrasound for the first time. The ultrasound technique represents a noninvasive dentin surface treatment and a conservative alternative to the removal of dental tissue that would promote groove formation on the dentin surface. It is a practical, fast, and easy procedure for experienced personnel. At 36 months, ultrasound treatment presented more marginal discoloration than did increased acid etching. The absence of improvement when using ultrasound could perhaps be attributed to it not removing the hypermineralized layer of the dentin surface. This dentin surface treatment needs to be further investigated in *in vitro* studies, and especially in longitudinal clinical evaluations.

Postoperative sensitivity decreased significantly in all experimental groups from the preoperative to the postoperative stage and was stable after the 36-month evaluation period. NCCLs can limit feeding in patients because the dentin tubules are open and exposed to the buccal environment, thereby causing various degrees of sensitivity. Sensitivity in the cervical region is predominant in the premolars,[46] as was also observed in our study. This is common in NCCLs, because the exposed dentin allows the movement of dentinal fluid that stimulates the nerve fibers of the pulp, causing pain. The treatments basically involve the obliteration of dentin tubules; however, the pain does not cease completely. In fact, pain is only reduced,[46,47] as was observed in this study, since teeth presented a reduction in sensitivity. The decrease in pain was possibly due to the obliteration of the tubules promoted by the composite used to restore the lesions.

Regarding marginal discoloration, all groups showed high marginal markings over time after a six-month period. Excessive or deficient filling materials at the margin forming gaps[48] and the retention of microscopic pigments derived from colored beverages and food in the adhesive layer can increase marginal discoloration.[48] Marginal staining has been thought to be one of the first clinical signs of failure of a composite restoration.[13] However, this marginal deterioration could not be considered a clinical failure. Restorations that lack marginal adaptation or marginal discoloration were

classified as clinically good or very good in other studies,[49,50] since these defects could be easily removed by finishing and polishing.

Previous studies[49,50] have suggested the possibility of establishing criteria that predict short-term restoration failure, ranging from postoperative sensitivity to loss of restoration. According to the guidelines of the American Dental Association,[51] the performance of adhesive systems is considered satisfactory if at least 95% of the restorations are retained after six months of evaluation. In this study, the number of restorations retained through 36 months of evaluation exceeded 95%. Therefore, it is possible to obtain good clinical performance in a relatively short period of time. One study[24] compared the six-month clinical behavior of several adhesion strategies using both FDI and United States Public Health Service (USPHS)–modified criteria. The findings suggested that the FDI criteria are more sensitive than the USPHS-modified criteria to small variations in clinical outcomes when evaluating restorations of NCCLs. Thus, we used the FDI criteria to assess the clinical outcomes related to composite restorations of NCCLs.

Additionally, some limitations of this study must be addressed, because randomized clinical trials are highly controlled clinical studies that include patients with specific characteristics to answer a specific research question, which would otherwise be clinically difficult for clinicians to address. It is important to consider that the results of our clinical trial are limited to the materials and techniques tested, as well as other individual factors that could modulate the longevity of experimental treatments. In addition, evaluations with longer follow-up periods might yield different results with surface treatment in NCCLs. Moreover, single studies provide very low-quality evidence suggesting rubber dam use in dental direct restorative treatments might improve the clinical success rate of restorations.[52] Although relative isolation was used in our clinical trial, a previous study[53] indicated that no significant differences were found between the types of isolation (absolute with rubber dam or relative) in composite Class V restorations of NCCLs. Future studies that evaluate dentin surface treatments with longer follow-ups should be performed for testing other treatments strategies, such as the use of a self-etch adhesive system with ultrasound or a longer acid-etching time only in sclerotic dentin. This may lead to the optimization of the clinical procedure for Class V composite restorations. Nevertheless, it is important to consider not only the restorative materials used in NCCLs but also the development and refinement of techniques that could improve the clinical longevity of composite restorations of NCCLs.

CONCLUSIONS

The dentin surface treatments evaluated in this study, such as longer acid-etching time and ultrasound, showed similar clinical performance to the conventional adhesive procedure up to the 36-month evaluation period, when considering marginal discoloration, marginal adaptation, and postoperative sensitivity. Increasing the acid-etching time also increased the risk of failure over time. Moreover, all dentin surface treatments reduced postoperative sensitivity over time.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the UFPel. The approval code for this study is 035-2011.

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.

(Accepted 17 May 2018)

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Clinical Evaluation of Two Different Prevention Programs in Adults Depending on Their Caries Risk Profile: One-year Results

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Clinical Relevance

The application of preventive protocols in patients with high and moderate caries risk differs. Regarding low-risk patients, the application of oral hygiene instructions is essential.

SUMMARY

The aim of this study was to investigate the management of incipient caries lesions in adults with two preventive protocols. A total of 44 adult patients with high, moderate and low caries risk with 516 incipient caries took part in the study. These patients were assessed for caries with International Caries Detection and Assessment System (ICDAS) criteria and were then divided into three groups depending on their caries risk profile: a high-risk group (group A), a moderate-risk group (group B), and a low-risk group (group C). Participants in each group were further divided randomly into two subgroups. In subgroups A1, B1, and C1, an intensive preventive protocol was applied, while in subgroups A2, B2, and C2, the protocol consisted only of instructions in oral hygiene. The invasive-intensive protocol included the topical application of fluoride, brushing with 5000-ppm fluoride toothpaste, use of amorphous calcium phosphate-casein phosphopeptide, applications of sealants for occlusal lesions (ICDAS code 2), and minimal resin restorations for occlusal lesions (ICDAS code 3). There was no statistically significant difference in the number of lesions (baseline and after one year) in the high-risk and moderate-risk groups that received the intensive protocol (groups A1 and B1), while the control groups were statistically significant different (groups A2 and B2). In the low-risk group, there was no statistically significant difference in the number of lesions (groups C1 and C2). The two different preventive protocols in the high- and moderate-risk groups presented dif-

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DOI: http://doi.org/10.2341/17-164-C

ferences in effectiveness, while in the low-risk group, no significant difference was demonstrated.

INTRODUCTION

The principles of modern caries management include detection and long-term monitoring of incipient noncavitated caries lesions, evaluation of the patient's caries risk, and efforts to reduce the risk, as well as caries management, by applying less invasive treatment options and always according to the caries risk profile of each patient.[1-3]

The treatment of patients should focus mainly on prevention (using high-fluoride toothpastes, solutions, patient education, and so on) and avoid further invasive treatments, thus achieving the goal of modern dentistry, which follows the philosophy of minimal intervention.[1] The effort of balancing the causative factors of caries with the protective factors, in conjunction with caries risk assessment, offers the ability to focus on the patient for prevention and treatment before irreversible lesions occur in dental tissues.[4]

For the application of modern caries management and treatment today, it is necessary to clinically apply a proper cost-effective prevention program on incipient caries lesions, according to minimally invasive principles, that guides the dentist beyond theory in clinical practice. The application of such a protocol to manage incipient caries lesions allows the dentist to stabilize and reverse incipient lesions with minimal "sacrifice" of healthy dental tissues.

Preventive programs on caries lesions in the adult population have been applied since 1970 and include various preventive measures, such as intensive topical application of fluoride, oral hygiene instructions, patient education and advice, and monitoring of lesions, all of which have been effective in reversing and preventing lesions for the past 40 years.[5-8]

In the dental literature, there are reports of clinical protocols that propose procedures to be followed to avoid caries. Even though such clinical protocols have been proposed, they have been poorly evaluated clinically in adult patients with different caries risk presenting with incipient lesions. Reports of such protocols' preventive programs have been published for adults, children, and adolescents.[9-12]

Single preventive measures on incipient caries in the dental literature have been evaluated, such as the application of sealants,[13-16] fluoride,[17-19] and amorphous calcium phosphate-casein phosphopeptide (ACP-CPP).[20-23]

With regard to preventive programs, few studies have evaluated protocols (but not for incipient caries), mainly on special needs patient groups, such as the elderly population,[24,25] patients with xerostomia,[26-28] patients who have undergone head and neck radiation and chemotherapy,[24,29-33] and other studies which have not evaluated incipient caries.[8,12] Additionally, few studies exist that refer to the patient's caries risk.

The purpose of this clinical study was to evaluate different protocols to treat incipient caries lesions in adults. These protocols were based on their caries risk profiles. Patients received dental treatment immediately after joining the study. The research hypothesis was that the applied protocols, depending on caries risk assessment of patients, do not present any difference. In particular, there is no difference in the number of incipient lesions after the application of these caries protocols, nor is there a difference in the development of incipient lesions compared to the control group.

METHODS AND MATERIALS

This study was conducted on patients attending the postgraduate Department of Operative Dentistry at the National Kapodistrian University of Athens. Specifically, the study included new patients entering the Postgraduate Clinical Program of Operative Dentistry but also patients who have previously been treated in that clinic. The study was approved by the Committee of Ethics of the Dental School of the University (protocol number 170), and all patients who took part in the study were informed and gave their prior written consent.

Inclusion and Exclusion Criteria for the Participants

Selection criteria were for patients to be over 18 years old and to have at least 10 natural teeth, without restorations and cavities. All the different medical conditions of the patients were included, except for those who were pregnant, patients who were in cancer therapy at the time of the study, or those with systematic diseases or who had taken medicines that influence the saliva. Exclusion criteria also included totally edentulous patients and patients with crowns, bridges, and restorations in 50% of more of their dentition.

Table 1: *Caries Risk Classification Based on the Clinical Characteristics of the Patients*

Caries Risk Group		
Low Risk	**Moderate Risk**	**High Risk**
No caries lesions *and/or* No proximal caries exceeding D2 (by bitewing X-rays)	Caries lesions 1-3 (ICDAS II) ≤ 3 *and/or* Open cavity ≤ 1 *and/or* Proximal lesions D3 ≤ 1 (by bitewing X-rays)	Caries lesions with open cavity ≤ 2 *and/or* Proximal lesions D4 category < 1 (by bitewing X-rays) *and/or* Caries lesions 1-3 (ICDAS II) > 3 (by bitewing X-rays)

Abbreviation: ICDAS, International Caries Detection and Assessment System.

Baseline Evaluation

Patients were examined clinically with International Caries Detection and Assessment System (ICDAS II) criteria[34] by one examiner and were then classified based on their caries risk profile into low-, moderate-, and high-risk groups. The evaluation of caries risk was based on the clinical features of caries risk, as shown in Table 1.

Teeth were coded from 0 to 6 according to the ICDAS II criteria (Table 2).[34,35] Only incipient lesions that were coded ICDAS 0-3 took part in the study, while more extended lesions (into dentin) that were coded ICDAS 4-6 were restored prior.

Each tooth surface was examined and scored under wet- and dry- conditions (air for five seconds) as per the ICDAS II instructions. The clinical examination of the teeth was made midday in a dental unit using the light and the air syringe of the unit by one examiner. The examiner was trained with educational software (ICDAS training software) to use these criteria before the clinical examination of the teeth. Further training was accomplished with a second examiner (also trained and familiar with the ICDAS II criteria). The two examiners were calibrated by pilot scoring 10 teeth independently and then seeking agreement for the scores presenting disagreement. After one week, scoring of 10 other teeth was repeated separately by the two examiners, and the two examiners came to full agreement. Finally, at a different time, the two examiners independently scored 38 teeth, and the agreement was substantial (Table 3).

During the patient's baseline clinical examination, all lesions with code 1, 2, and 3 based on the ICDAS II criteria were photographed by the examiner. For the baseline evaluation of the proximal lesions, digital radiography was used (Vista Scan device and DBSWIN software, Dürr Dental, Bietigheim-Bissingen, Germany).

Table 2: *International Caries Detection and Assessment System (ICDAS II) Codes With Direct Visualization of the Teeth First in Moisture and After Drying Them With Air for Five Seconds*[34,35]

Visual Examination ICDAS Codes	
0	Sound tooth surface
1	First visual change in dry enamel
2	Distinct visual change in moist enamel
3	Localized enamel breakdown due to caries with no visible dentine or underlying shadow
4	Underlying dark shadow in dentine with or without localized enamel breakdown
5	Distinct cavity with visible dentine
6	Extensive distinct cavity with visible dentine

Application of Protocols

Control Group—Patients in the control group were given only oral hygiene instructions to brush (Caries Protection toothpaste, Colgate fluoride, 1450 ppm, Colgate-Palmolive, Cincinnati, OH, USA) and floss interproximally (waxed dental floss was given to patients; Colgate). All patients (control and intervention groups) were instructed to brush with the Bass technique twice a day and to floss once per day, and for this purpose leaflets with these instructions were given. Additionally, they were instructed with demonstration models and trained with a toothbrush in their mouth. Lesions with the 1-3 ICDAS code were recorded and shown to the patients.

Table 3: *Interexaminer Agreement Between the Two Examiners in ICDAS Criteria (International Caries Detection and Assessment System) and Intraexaminer Agreement in Different Time Interval Examinations*

K (ICDAS)	K	Agreement
Examiners	0.73 ± 0.07	Substantial
Different time interval examination	0.74 ± 0.04	Substantial

Table 4: *General and Specific Measures Applied to the Patients in the Intervention Group*

Patients Intervention group		HIGH CARIES RISK A1				MODERATE CARIES RISK B1			LOW CARIES RISK C1		
Treatment: General measures		Enhanced oral hygiene instructions with 5000ppm F⁻toothpaste				Enhanced oral hygiene instructions with 5000ppm F⁻toothpaste			Oral hygiene instructions		
		Dietary analysis and advice				Dietary advice			Dietary advice		
		Topical application of fluoride (1.23% NaF gel[a]) or CPP-ACP[b], 1 time/3 months				Topical application of 1.23% NaF gel[a], 1 time/every 6 months			Topical application of fluoride(1.23% NaF gel[a]), 1 time/year		
		Pit and fissures sealants for teeth with deep grooves (mandatory)				Pit and fissures sealants for teeth with deep grooves (optional)					
		Recall, 3 months				Recall, 6 months			Recall, every year		
Treatment: specific measures for each lesion		CPP-ACP[b]	seal ants	ICON	Resin restor ation	CPP-ACP[b]	Sealan ts	Resin restora tion	CPP-ACP[b]	sealants	Resin restor ation
Lesion		High caries risk				Moderate caries risk			Low caries risk		
Occlusal	01 ICDAS										
	02 ICDAS		√				√				
	03 ICDAS				√			√		√	
Smooth surfaces	01 ICDAS	√				√					
	02 ICDAS	√		√ anterior teeth		√					
	03 ICDAS				√	√			√		
Proximal	01 ICDAS	√				√			√		
	02 ICDAS	√				√			√		
	03 ICDAS				√			√	√		
Root caries	1 ICDAS				√	√					

[a] *C Care, 1.23% NaF gel, APF (Dental Line Ltd, Athens, Greece).*
[b] *Amorphous calcium phosphate-casein phosphopeptide (ACP-CPP) cream, GC Tooth Mousse (GC Corp, Tokyo, Japan).*

Intervention Group—In this group, in lesions coded 1, 2, and 3 according to ICDAS II criteria, protocols of caries management were applied. Protocols were applied by the same examiner who conducted the evaluations. Protocols (presented in Table 4) included general preventive measures depending on the patient's caries risk, followed by specific measures for each lesion.

Evaluation Stage—In the control and intervention groups, recalls were performed at time intervals according to the caries risk of each patient. High-risk patients were re-examined every three months, moderate-risk patients every six months, and low-risk patients at one year. At the recalls, each lesion, treated or not, was re-evaluated by the clinical ICDAS criteria (pit and fissure caries, root caries, caries on smooth surfaces, and proximal surfaces). At the recall, the percentages of lesions that had further developed or stabilized were calculated. Finally, each patient was examined for the occurrence of new lesions. Additionally, comparison of the treatment and control groups was performed.

Sample

A total of 50 patients took part in the present study (n=50); however, six patients withdrew from the study before the one-year recall. The final sample that was evaluated consisted of 44 people (9 men and 35 women) aged 20-62 years old with a mean age of 30 years. Before the final selection of the patients, a pilot study was performed with three patients of high caries risk, and the power of the results was higher than 0.8. The results deemed to be clinically relevant and to have that high power were the differences between the number of caries in these three patients at two separate visits. After the ICDAS II examination of the patients and the evaluation of their caries risk, the randomization process was as follows: the first patient who took part in the study of each patient risk group was included in the intervention group and the second in the control group and the sequence continued in this way.

Statistical Methods

Findings were analyzed as follows:

1) The percentage and absolute number of incipient lesions in all groups at baseline examination and at the one-year recall as well as the $D_{1\text{-}3}$MFS index for each group were calculated ($D_{1\text{-}3}$: for each participant the incipient lesions with 1-2-3 ICDAS code).
2) The changes for each lesion from the baseline examination to the one-year recall were recorded. The nonparametric Wilcoxon test was applied to the two visits (baseline and one year) in each group for comparing the number of lesions. Computer software (SPSS Statistics version 13.7, IBM, Armonk, NY, USA) was used, and the level of statistical significance was 0.05.

RESULTS

Percentage and Absolute Number of Incipient Caries in All Groups at the Baseline Examination and the One-Year Recall by Caries Risk

The results are presented in Tables 5-7.

DMFS Index for Each Group- Wilcoxon Test

The $D_{1\text{-}3}$MFS index for each group is presented in Table 8.

The Wilcoxon test (Table 8) revealed statistically significant differences in the high-risk control group (group A2, p=0.024) with the DMFS index decreasing from 16.83 ± 1.00 (mean±SE) to 14 ± 1.15 (mean±SE) after one year and in the moderate risk control group (group B2, p=0.041) with the DMFS index increasing from 12.43 ± 1.47 (mean±SE) to 14.7 ± 1.82 (mean±SE) after one year. There was no statistically significant difference in the number of lesions among the high-risk and moderate-risk patient groups when preventive protocols were applied (group A1, p=0.786; group B1, p=0.233). In the low-risk group, there was no statistically significant difference in the number of lesions (group C1, p=0.203; group C2, p=0.303).

DISCUSSION

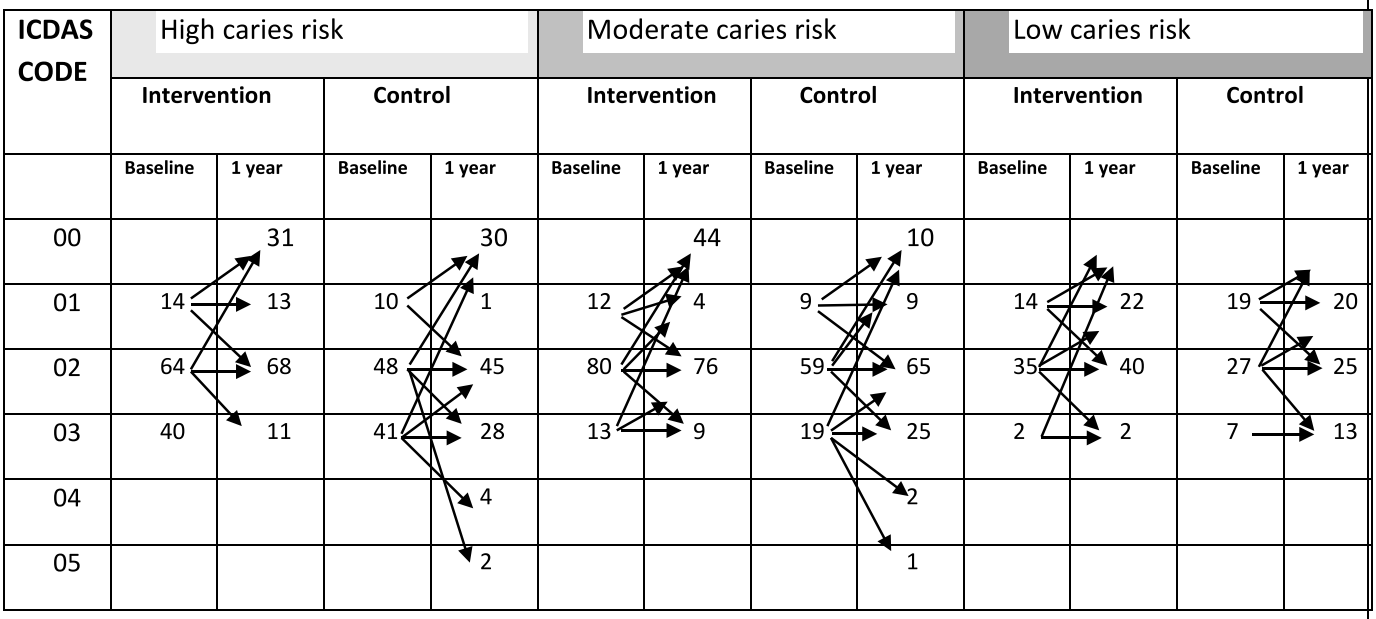
The protocols for the management of incipient caries lesions evaluated in this clinical study include preventive and minimally invasive methods as published in the most recent studies of incipient lesions treatment. Different protocols were applied based on the caries risk of each patient. All caries were identified using the latest modified version of the ICDAS criteria (ICDAS II).[34,35]

In the present study, the caries risk of each patient was determined based solely on the clinical examination and the existing number of lesions. Due to the complexity and difficulty of applying the various caries risk evaluation strategies (because of the multiple collection of data), research tends to simplify this procedure so that it can be incorporated

Table 5: *Breakdown Results to Participant in Baseline Examination and One-Year Recall*

Patient		Baseline	1 year
1	High caries risk	32	26
2	Intervention group	24	19
3		12	13
4		18	23
5		20	24
6		12	12
1	Control group	17	15
2		18	12
3		21	19
4		16	14
5		14	11
6		15	13
1	Moderate caries risk	22	23
2		17	10
3	Intervention group	12	10
4		17	16
5		14	18
6		10	9
7		13	6
1	Control group	11	16
2		14	19
3		19	21
4		14	17
5		9	10
6		7	8
7		13	12
1	Low caries risk	4	10
2		5	5
3	Intervention group	11	14
4		5	4
5		6	3
6		6	9
7		7	6
8		2	3
9		9	14
1	Control group	6	6
2		5	8
3		3	5
4		5	8
5		7	4
6		6	7
7		0	1
8		10	9
9		10	10

Table 6: *Baseline Incipient Lesions and One-Year Recall Regarding the Changes of the Incipient Lesions With International Caries Detection and Assessment System (ICDAS II) Criteria*[a]



[a] *Arrows show each ICDAS code before and after intervention for each group.*

easily into the everyday clinical dental practice of caries risk assessment. In a retrospective analysis by Domejean and others,[36] who evaluated the CAMBRA system in 2571 patients, it was concluded that "visible cavities" and cavities with "radiographic penetration into dentin" were significantly associated with the caries risk. Another study evaluated Cariogram and suggested that it is possible for the evaluation of caries risk to find more simplified models using regression analysis for the determination of risk.[37] In another study, Bader and others[38] ranked 45,693 patients, indicating a way to classify each individual by caries activity at the time of examination. This classification integrates and unifies the entire patient history and is associated with patient behavior and caries risk.[38] Later, Bader and others[39] assessed the subjective evaluation of the dentist in determining caries risk, what additional information dentists examine, and if there is a need for direct involvement of the dentist in caries risk assessment. The subjective evaluations of dentists contributed to further increase of risk evaluation sensitivity. In conclusion, the determination of caries risk based on current dental status is well documented and approaches the clinical reality.[39]

Table 7: Baseline Number of Patients and Lesions and One-Year Recall Regarding the Changes of the Incipient Lesions (Numerically and %) in High-, Moderate-, and Low-Risk Patients

Caries Risk Group	High-Risk Patients		Moderate-Risk Patients		Low-Risk Patients	
	Intervention	Control	Intervention	Control	Intervention	Control
Patients (number)	6	6	7	7	9	9
Baseline examination (number of lesions)	118	99	105	87	55	52
1-y recall/lesions						
Increased number of caries (%)	9 (8%)	9 (8%)	7 (5%)	17 (16%)	5 (6%)	9 (14%)
Decreased number of caries (%)	32 (29%)	45 (39%)	48 (37%)	15 (14%)	18 (22%)	13 (20%)
Stabilized number of caries (%)	38 (35%)	45 (38%)	45 (34%)	55 (50%)	31 (38%)	30 (46%)
New caries number of caries (%)	31 (28%)	18 (15%)	31 (24%)	22 (20%)	28 (34%)	13 (20%)

Table 8: *DMFS Index in the Intervention and Control Groups of High-, Moderate-, and Low-Risk Patients and Wilcoxon Test Statistics for Comparison Among Groups in Number of Lesions at Baseline and in One Year*

DMFS	DMFS					
	High Caries Risk		Moderate Caries Risk		Low Caries Risk	
	Intervention Group A1	Control Group A2	Intervention Group B1	Control Group B2	Intervention Group C1	Control Group C2
Baseline	19.66 ± 3.11 (mean±SE)	16.83 ± 1.00 (mean±SE)	15.00 ± 1.50 (mean±SE)	12.43 ± 1.47 (mean±SE)	6.11 ± 0.88 (mean±SE)	5.77 ± 1.00 (mean±SE)
Year	19.50 ± 2.40 (mean±SE)	14.00 ± 1.15 (mean±SE)	13.14 ± 2.27 (mean±SE)	14.70 ± 1.82 (mean±SE)	7.55 ± 1.46 (mean±SE)	6.44 ± 2.78 (mean±SE)
Wilcoxon *p*	$p = 0.786$	$p = 0.024$	$p = 0.233$	$p = 0.041$	$p = 0.203$	$p = 0.303$

In the present study, in patients with high or moderate caries risk, the incipient caries lesions with ICDAS code 3 were restored with resin composite after minimal preparation of these lesions. A recent systematic review of 14 studies, including 1440 patients with 3551 lesions, found that conservative treatment (minimal invasive) of incipient lesions required less retreatment compared to lesions that were not treated. The researchers also concluded that the strategy of minimally invasive treatment was the most effective, followed by microinvasive and noninvasive treatment.[40]

In the present study, a statistically significant difference with regard to the number of caries lesions was found only for the control groups of high- and moderate-risk patients. In the other groups, where the preventive protocols were applied, no statistically significant differences were found. Intervention groups of high- or moderate-risk patients were not found to be statistically significant different, although the protocols were quite intensive.

There are no data from studies of the management of incipient caries in adults. For this reason, the results of the present study were compared with relative studies conducted with children. This is not really clinically relevant, but it does present a view of what happens in different age-groups. The results of the aforementioned study could be in agreement with a study that treated high caries risk children with sealants and supervised brushing. In this study, daily supervised brushing was as good as the application of sealant materials in preventing lesions from advancing to cavities.[15] In contrast to the above, Borges and others[41] evaluated the influence of sealants in patients with high or moderate caries risk and concluded that the measure is effective in reversing lesions.

Although the results of these studies[15,41] conflict with those of the present study, they reach similar conclusions. Statistically significant differences were found in the control groups of high- and moderate-risk patients but, when evaluated together with the changes in the DMFS index of these two groups, have opposite results. In high-risk patients, the DMFS index decreased from 16.83 to 14.00 solely by emphasizing oral hygiene (meaning that oral hygiene was effective for this patient group, as the number of carious surfaces decreased). However, the opposite was found for the control group of moderate-risk patients, as the DMFS increased from 12.43 to 14.70 in one year; thus, oral hygiene proved an insufficient measure for these patients as the number of carious surfaces increased.

In the present study, in the intervention group of high-risk patients, no statistically significant difference was found in the recall at one year with regard to the number of caries lesions, and the DMFS index remained stable (from 19.50 to 19.60 in one year). However, in the control group, solely through meticulous oral hygiene, a statistically significant difference was found in the number of lesions, and the DMFS index decreased from 16.83 to 14.00.

Results of the present study are in disagreement with other studies where toothpaste that contains 5000-ppm fluoride was significantly better compared to toothpaste that contains 1450-ppm fluoride in reversing incipient lesions without cavity formation.[42-44] The comparison of the present results with previous studies is difficult, and Bader and others[45] reached the same conclusion in a systematic review of 1435 articles on the effectiveness of preventive methods in high-risk individuals with incipient lesions. They concluded that the main limitations of the literature regarding the treatment of incipient lesions were the small number of studies as well as variation among patients, tooth surfaces, caries

assessment and lesion progression criteria and variation in the experimental and control groups; thus, it is impossible to achieve a comparable conclusion about the effectiveness of any method.

In patients with moderate caries risk in the intervention group, no statistically significant difference was found in the number of lesions before and after the application of the prevention program. The DMFS index decreased from 15.00 ± 1.50 on the baseline examination to 13.14 ± 2.27 at the one-year recall. In the control group, the DMFS increased from 12.43 to 14.70 at one year and had a statistically significant difference (p=0.041).

Pit and fissure sealants materials have been found protective in adolescents, but only in low- or moderate-risk patients.[46] Unfortunately, in the literature, studies related to moderate-risk patients are largely missing. Based on all this, a reliable conclusion cannot be drawn from the comparison of the results of the present study for this caries risk group.

In the low-risk group, there was no statistically significant difference in any of the treatment or control groups in the number of lesions. Studies in children and adolescents concluded that this measure is clearly effective. However, for sealants, cost-effectiveness must be taken into serious consideration in low-risk groups because there are other measures, such as the use of fluoride and oral hygiene instructions, that could achieve the same results.[47,48]

Moreover, in the present study, in the high-risk patients, the intervention and control subgroups had the same increasing rate of caries lesions (8%), while there was a difference in the intervention group in the occurrence of new lesions (28%) and a reduction of existing lesions (29%) compared with the control group, where the percentages were 15% (new caries) and 39% (reduced caries), respectively. However, in the present study, in the annual recall in the intervention group after the application of the protocols, there were no lesions greater than ICDAS code 3. That means that in this group, the caries lesions remained in the enamel, while in the control group, some lesions progressed further into the dentin since four lesions were identified as ICDAS code 4, one lesion was identified as ICDAS code 5, and a composite resin restoration was placed in a previous recall.

This final result of the present study agrees with the two-year study of Liu and others[17] of 501 patients who applied three different preventive measures in incipient occlusal caries lesions in 1.491 molars with lesions code 2 according to the ICDAS criteria. After two years, dentin lesions in the control group were 4.6% and were statistically significantly higher than in the other three treatment groups (p=0.002).

Regarding the moderate-risk patients, in the intervention group, 37% of the lesions decreased, while only 5% increased. In the control group, 14% of the lesions decreased, while 16% increased. The application of the protocol in patients with moderate caries risk presented differences compared with the control group.

In the low-risk patients in the intervention group, 6% of the lesions increased, 34% new lesions developed, and 38% remained stable, while in the control group, the corresponding rates were 14% (increased), 20% (new caries), and 46% (remained stable).

The duration of the present study was one year. The intensive preventive programs clearly had no favorable results at this one-year period. Longer periods of observation and intervention are probably needed to show effectiveness.

CONCLUSIONS

- In patients with moderate caries risk, the intervention group presented lower caries development rates and a lower DMFS index than the control group; thus, oral hygiene instructions given to the control group are not adequate to control caries.
- In patients with high caries risk, the result of this study is not clear. The control group presented significantly lower DMFS than the intervention group. The control group presented overall better results than the intervention group; however, some incipient lesions further progressed into dentin in the control group, a fact that did not exist in the intervention group. Thus, in high-risk patients due to the above-mentioned result, the dentist must choose whether to apply to the patient an intensive protocol; however, the risk of some incipient lesions to progress into dentin must be considered. In such cases, the final selection should be done in collaboration with the patient, and cost-effectiveness should be taken into account.
- Regarding the low-risk patients, both protocols proved to have no difference in results, meaning that in these patients, the application of only oral hygiene instructions is adequate for the management of incipient lesions.

- Incipient lesions in patients should be monitored regularly, as the present study showed that these lesions may advance in less than a year to a "worse" category (as evaluated with ICDAS II criteria).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Committee of Ethics of the Dental School of the University. The approval code for this study is protocol number 170.

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.

(Accepted 24 January 2018)

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Comparison of Whitening Dentifrices on the Effectiveness of In-office Tooth Bleaching: A Double-blind Randomized Controlled Clinical Trial

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Clinical Relevance

The use of whitening dentifrices is not recommended during in-office tooth bleaching. However, the application of whitening dentifrices after the bleaching treatments helps to maintain the whitening results.

SUMMARY

Objectives: To investigate the effect of whitening dentifrices on the effectiveness of in-office tooth bleaching.

Methods and Materials: A double-blind randomized controlled clinical trial was performed. The participants were randomly allocated into three groups according to the different dentifrices used during this clinical trial: regular dentifrice (group C), conventional whitening dentifrice (group CW), and whitening dentifrice containing blue covarine (group CU). All participants received in-office tooth bleaching for the maxillary anterior teeth (two sessions conducted at a one-week interval). Tooth color was measured with a spectrophotometer at baseline (T1), after the first bleaching session (T2), after the second bleaching session (T3); one week after the completion of in-office bleaching (T4); and three weeks after the completion of in-office

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DOI: http://doi.org/10.2341/17-333-C

bleaching (T5). The data were statistically analyzed through repeated analysis of variance and the Tukey test (α=0.05).

Results: Sixty participants completed the study (n=20 per group). At T3, group CU exhibited the lowest ΔE values (p=0.008). The ΔE values increased from T4 to T5 in the CW and CU groups, whereas a decrease in ΔE values was observed for group C.

Conclusions: The use of a whitening dentifrice containing blue covarine during in-office bleaching reduced color changes. After tooth bleaching, brighter tooth colors were observed in the participants who brushed with whitening dentifrices compared to those who brushed with a regular dentifrice.

INTRODUCTION

With increasing aesthetic demands from patients, tooth whitening has become a popular treatment option for creating whiter and brighter smiles.[1-3] Currently, there are three tooth whitening approaches: at-home tooth bleaching, in-office tooth bleaching, and over-the-counter (OTC) whitening products.[4] In-office and at-home bleaching approaches are considered well-established procedures in cosmetic dentistry due to their effectiveness and safety.[5-7] Compared with at-home bleaching, in-office bleaching, which is usually performed with relatively high concentrations of hydrogen peroxide/carbamide peroxide, is more efficient and performed in weekly sessions.[8] However, in some studies, in-office bleaching has demonstrated relatively lower posttreatment color stability than at-home bleaching.[9]

In addition to in-office and at-home bleaching procedures, OTC whitening products, including whitening dentifrices and mouth rinses, have garnered increasing attention from consumers.[10] Various whitening dentifrices are currently available on the market. The components of whitening dentifrices include fluoride, pyrophosphates, triclosan, potassium nitrate, and even peroxide at low concentrations.[11] A recent systematic review concluded that whitening dentifrices demonstrate beneficial effects in reducing extrinsic tooth staining regardless of the presence of peroxide.[12] However, in terms of overall tooth color changes, previous studies have provided contrasting results regarding the effects of whitening dentifrices. In fact, different studies have reported ΔE values ranging from 0.3 to 5.1 after patients brush with the whitening dentifrices for a certain period of time.[13-17] This controversy might be explained by variations in the study protocols and the composition of the whitening dentifrices. It is only through their abrasive nature that most contemporary whitening dentifrices aid the removal of extrinsic tooth stains. Abrasiveness is influenced by particle hardness, shape, and size as well as the pH of a dentifrice.[11] Other whitening dentifrices, containing peroxide and enzymatic agents, can release free radicals to break down the chromogens of discolored teeth.[13] Moreover, whitening dentifrices containing blue covarine can change the optical properties of teeth and increase tooth whiteness.[10]

Based on the stain removal/prevention capability of a tooth whitening dentifrice, it could be postulated that the whitening dentifrice and professional bleaching treatments have synergetic effects on the final appearance of the teeth. In a laboratory study, Bortolatto and others[18] compared the efficacy of whitening dentifrices and placebo dentifrices after professional tooth bleaching treatments. The whitening dentifrices failed to provide beneficial effects on teeth previously bleached using professional bleaching treatments. Vieira-Junior and others[19] reported that the application of regular dentifrice prior to tooth bleaching has no influence on the effectiveness of bleaching treatment. Moreover, brushing with whitening dentifrices during professional bleaching procedures might increase the surface roughness of the enamel.[11,20] However, there is limited information regarding the effects of whitening dentifrices on the effectiveness of professional tooth bleaching treatments.

Therefore, this double-blind randomized controlled clinical trial aimed to determine whether whitening dentifrices would improve the effectiveness of in-office tooth bleaching procedures. The following null hypotheses were tested: 1) the use of whitening dentifrices during an in-office bleaching treatment would produce similar tooth color changes as regular dentifrice, and 2) the use of whitening dentifrices after completion of an in-office bleaching treatment would produce similar tooth color changes as regular dentifrice.

METHODS AND MATERIALS

This study was approved by the ethics committee of the local university and performed following the Consolidated Standards of Reporting Trials guidelines[21] (Figure 1), consistent with the principles of good clinical practice of the Declaration of Helsinki. This study was registered in the Clinical Trials Registry (http://www.ClinicalTrials.gov).

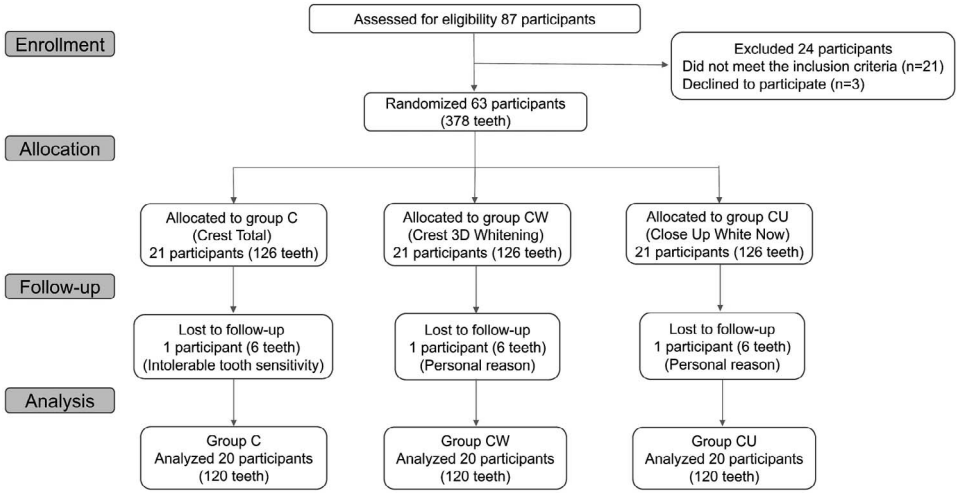

Figure 1. *Study flow diagram.*

Study Design

This study was a double-blind randomized controlled clinical trial and conducted at the Hospital of Stomatology of the local university from January 2017 to August 2017.

Inclusion and Exclusion Criteria

The participants who applied to the study were examined and selected based on the following inclusion criteria: 1) 18 to 60 years of age and in good general health; 2) six fully erupted maxillary anterior teeth, with no oral disease or dental restorations; and 3) at least one maxillary tooth demonstrating shade A3 or darker, as measured with Vita Easyshade Advance 4.0 (Vita Zahnfabrik, Bad Säckingen, Germany) (ordered by brightness). The following exclusion criteria were applied: 1) systemic diseases or oral mucosal disorders, 2) previous bleaching treatments, 3) current orthodontic treatment, 4) pregnancy, 5) known allergies to the product ingredients, 6) smoking, 7) alcohol abuse, and 8) gingival recession.[22]

At the screening visit, the selected participants gave their written informed consent to participate in the study.

Sample Size Calculation

The primary study outcome (ΔE values) was considered the variable used for sample size calculation. The sample size was calculated to obtain a ΔE difference of one unit at the end of the study between experimental group and control group.[15,23] This difference was determined by a pilot study considering the maximum standard deviations obtained. A power analysis was conducted, with 80% power at an alpha level of 0.05 for a two-tailed test. The power calculation showed that a sample size of 19 per group was necessary to detect significant differences among the groups. Assuming an anticipated dropout rate of 10%, the final sample size was defined as 21 participants per group.

Study Intervention

During this four-week clinical trial, all participants underwent two sessions of in-office bleaching treatment and were asked to use only the distributed dentifrices. A dental prophylaxis, including scaling and polishing, was performed for each participant before bleaching treatment. The polishing was finished with the aid of polishing cup and fine-grit polishing paste (Proxyt RDA 7, Ivoclar Vivadent, Schaan, Liechtenstein). Maxillary impressions of the participants were produced using alginate materials (Heraeus, Hanau, Germany), which were then poured in dental stone (Heraeus). In-office bleaching treatments were performed by a proficiency dentist who was trained and calibrated for this study using 40% hydrogen peroxide gel (Opalescence BOOST PF 40%, Ultradent, South Jordan, UT, USA). A light-cured resin barrier (OpalDam, Ultradent) was applied over the gingival tissue around the teeth to be bleached. The bleaching gels were freshly mixed and applied on the labial surfaces of the maxillary anterior teeth at a thickness of 1 mm. Two 30-minute applications were performed per session, and the bleaching gels were refreshed after each application. The bleaching sessions were performed at room temperature at a one-week interval.

Two whitening dentifrices (Crest 3D Whitening, Procter & Gamble, Blue Ash, OH, USA; Close Up White Now, Unilever, São Paulo, Brazil) and one

regular dentifrice (Crest Cavity Protection, Procter & Gamble) were used in this study. The eligible participants were randomly allocated into three groups according to the dentifrices used in this trial (n=21 per group): group C (Crest Cavity Protection, a regular dentifrice), group CW (Crest 3D Whitening, a conventional whitening dentifrice), and group CU (Close Up White Now, a whitening dentifrice containing blue covarine).

For allocation of the participants, a computer-generated list of random numbers was used. One of the investigators who was not involved in the clinical section of the study transferred the dentifrices from their original tubes to identical soft opaque tubes and labeled the tubes with numbers for each participant according to the randomization schedule. After the research assistant had obtained the participant's consent, he delivered the tubes containing dentifrices (enough for four weeks of use) to the participants together with a standard soft-bristled toothbrush (Crest, Procter & Gamble). In this manner, both the investigators and the participants were blinded to the dentifrices being delivered.

The participants were instructed to brush their teeth twice daily for two minutes each time using a Modified-Bass method. All participants were asked to avoid tobacco use and staining foods and drinks, such as curry, cola, coffee, and so on, during the experimental period. Moreover, all participants were asked to report any adverse reactions associated with bleaching, and the adverse reactions were addressed accordingly.

Evaluation

The colors were measured with CIE L*a*b* color space using a spectrophotometer (Vita Easyshade Advance 4.0). For the color measurement, a customized plastic tray with six location cones (6 mm in diameter) for each participant was fabricated. Therefore, the color measurement can be standardized by positioning the tip of the spectrophotometer with the location cones on the middle third of the labial tooth surfaces before and after each treatment. The spectrophotometer was calibrated for each subject's measurement. Tooth color was assessed at the following time points: baseline - prior to the bleaching treatment (T1), immediately after the first bleaching session (T2), immediately after the second bleaching session (T3), and one week (T4) and three weeks (T5) after the completion of the bleaching treatment. At all color measurement points, three measurements were performed for each tooth, and the average values of L*, a*, and b* were calculated for statistical analysis.

The ΔE and whiteness index (W) were further calculated using the following formulas:[24]

$$\Delta E = \sqrt{(L_i^* - L_0^*)^2 + (a_i^* - a_0^*)^2 + (b_i^* - b_0^*)^2} \quad (1)$$

and

$$W = 100 - \sqrt{[(100 - L_i^*)^2 + a_i^{*2} + b_i^{*2}]} \quad (2)$$

where L* refers to the lightness from black to white, a* refers to the color along the red and green dimension, and b* refers to the color along the yellow and blue dimension. The subscript letter "i" refers to the measurements at each testing interval, and "0" refers to the baseline measurements.

Statistical Analysis

The assumption of an approximately normal data distribution was confirmed using the Kolmogorov-Smirnov test. The L*, a*, b*, W, and ΔE values at each testing interval were analyzed using two-way repeated-measures analysis of variance to evaluate the effects of the dentifrices and testing time intervals (as a repeated measure). The data were analyzed using the SPSS statistical software package (SPSS 19.0 for Windows, SPSS, Chicago, IL, USA). All statistical analyses were performed at a significance level of 0.05.

RESULTS

Eighty-seven volunteers were screened for eligibility, 63 of whom (34 females and 29 males, mean age 26.1 years) were enrolled. Baseline and demographics data were similar across the three groups. One participant from group C declined to complete the study due to intolerable tooth sensitivity, and one participant from group CW and one from group CU were lost to follow-up for personal reasons. Sixty participants (n=20 per group) completed this clinical trial and were included in the analysis. During the treatment period, no significant adverse reactions were reported by the participants completing the study. Noticeable sensitivity was observed during and immediately after the bleaching treatment. The sensitivity usually disappeared one to two days after the bleaching treatment. Moreover, the presence of sensitivity was similar across the three groups.

Table 1: *Means and Standard Deviations of the Color Parameters (L*, a*, b*, and W) for All Groups at Different Time Points (T1 to T5)*[a]

Groups	T1	T2	T3	T4	T5
L* value					
C	74.95 (4.16) Aa	77.78 (4.09) Bb	79.56 (3.78) Dc	78.43 (4.25) Ec	78.60 (3.69) Fc
CW	74.71 (4.64) Ad	76.97 (4.75) Ce	79.61 (3.78) Df	78.27 (4.43) Eg	79.30 (4.02) Fg
CU	75.87 (4.79) Ah	77.43 (5.02) Bi	79.22 (4.16) Dj	78.42 (4.73) Ej	78.59 (4.40) Fj
a* value					
C	0.71 (1.29) Aa	0.40 (1.18) Bb	−0.78 (0.77) Cc	−1.17 (0.77) Dd	−1.06 (0.64) Fd
CW	0.62 (1.32) Ae	0.38 (1.28) Be	−0.79 (0.90) Cf	−1.07 (0.66) Dg	−1.12 (0.72) Fg
CU	0.67 (1.38) Ah	0.32 (1.18) Bl	−0.73 (0.68) Cj	−0.91 (0.83) Ej	−1.09 (0.71) Fk
b* value					
C	18.16 (4.44) Aa	17.38 (4.10) Ba	12.77 (4.02) Cb	11.17 (3.48) Dc	11.39 (3.53) Ec
CW	17.55 (4.27) Ad	17.30 (4.19) Bd	13.32 (3.43) Ce	11.03 (2.99) Df	10.94 (3.32) Ef
CU	17.79 (4.45) Ag	16.79 (3.88) Bg	13.20 (3.11) Ch	11.51 (3.13) Di	11.41 (3.41) Ei
W value					
C	68.80 (4.85) Aa	71.62 (4.96) Bb	75.62 (4.09) Cc	75.38 (3.92) Dc	75.43 (3.46) Ec
CW	68.93 (5.02) Ad	70.98 (5.36) Be	75.38 (3.90) Cf	75.36 (3.96) Df	76.30 (4.01) Ef
CU	69.78 (5.13) Ag	71.64 (5.39) Bh	75.11 (3.86) Ci	75.20 (4.08) Di	75.37 (3.78) Ei

Abbreviations: C, Crest Cavity Protection as a regular dentifrice; CW, Crest 3D White as a conventional whitening dentifrice; CU, Close Up White Now as a whitening dentifrice containing blue covarine.
[a] Values marked with the same uppercase letter were not significantly different at each time point among the three experimental groups for each color parameter (p>0.05). Values marked with the same lowercase letter were not significantly different in each group for each color parameter (p>0.05).

After bleaching, the tooth colors of the participants in all groups became whiter, with increased L* and W values and decreased a* and b* values (all $p<0.001$) (Table 1). The mean ΔE values were 8.19 for group C, 7.62 for group CW, and 6.90 for group CU. Significantly lower ΔE values were observed in group CU ($p=0.008$). After the bleaching treatment ended, an increase in the ΔE values (from T4 to T5) was observed in groups CW and CU, whereas a decrease was observed in group C (Table 2). A decrease in tooth yellowness (yellow to blue color shift, reduction in b* value) was observed in groups CW and CU, whereas an increase in b* value was recorded in group C from T4 to T5.

The ΔE values for different tooth notations were calculated by averaging the contralateral teeth (Figure 2). Significantly greater color changes were observed in the canines than in the central and lateral incisors (all $p<0.05$).

DISCUSSION

Based on the study findings, the null hypotheses that the use of whitening dentifrices during in-office bleaching treatment would produce similar tooth color changes as regular dentifrices and that the use of whitening dentifrices after the completion of in-office bleaching treatment would produce similar tooth color changes as regular dentifrices were rejected.

Over the past decade, OTC whitening products have become increasingly popular on the market. Whitening dentifrices containing abrasives, chemicals, or optical agents have demonstrated their ability to remove and prevent the formation of extrinsic stains.[10,12] However, limited evidence is available to determine whether the application of whitening dentifrices during professional bleaching treatment has effects on tooth color. The present clinical

Table 2: *Means and Standard Deviations of ΔE Values for All Groups at Different time Points (T2 to T5)*

Group	T2	T3	T4	T5
C	4.57 (2.51) Aa	8.19 (3.37) Cb	9.36 (3.05) Ec	8.85 (3.12) Gc
CW	4.19 (2.39) Ad	7.62 (2.80) Ce	8.88 (3.11) Ef	9.25 (3.73) Gf
CU	3.92 (2.20) Bg	6.90 (2.89) Dh	8.21 (3.29) Fi	8.26 (3.42) Hi

Abbreviations: C, Crest Cavity Protection as a regular dentifrice; CW, Crest 3D White as a conventional whitening dentifrice; CU, Close Up White Now as a whitening dentifrice containing blue covarine.
[a] Values marked with the same uppercase letter were not significantly different at each time point among the three groups for each color parameter (p>0.05). Values marked with the same lowercase letter were not significantly different in each group for each color parameter (p>0.05).

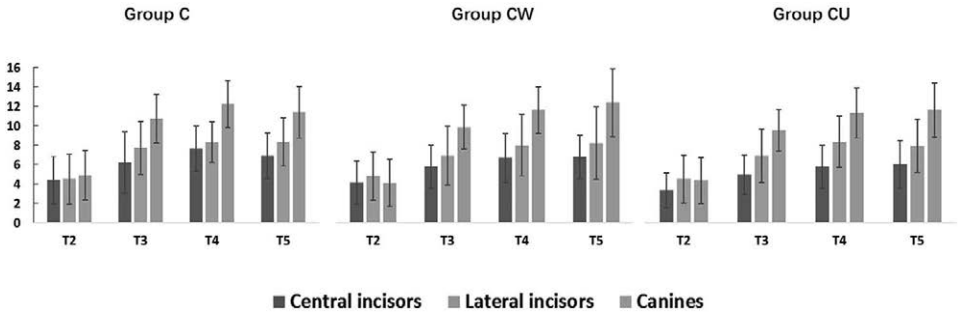

Figure 2. *ΔE values of different teeth at different time points in the three experimental groups. C, Crest Cavity Protection as a regular dentifrice; CW, Crest 3D White as a conventional whitening dentifrice; CU, Close Up White Now as a whitening dentifrice containing blue covarine.*

investigation can be considered the first clinical trial aiming to clarify the above-mentioned problem.

In the present study, groups CW and group C exhibited the same ΔE values after in-office bleaching, indicating that the tested whitening dentifrice and professional bleaching treatment had no synergistic effects on tooth color. More interestingly, group CU showed the lowest ΔE values after professional bleaching treatment (T3). The participants in group CU were asked to use a silica-based whitening dentifrice containing blue covarine. Previous studies have shown that the whitening effects demonstrated by dentifrices containing blue covarine are instantaneous, long lasting, perceivable, and measurable.[17,25] The mechanism of action is based primarily on the deposition of a thin film that is capable of altering the visual perception of the tooth color.[10] A characteristic peak of blue covarine, which contains a phthalocyanine ring structure with a tightly bound central copper ion, was observed on the enamel surface by time-of-flight secondary ion mass spectrometry.[26] This coating is deposited on the tooth surface after brushing, and it might inhibit the penetration of bleaching agents and further hinder the effectiveness of bleaching treatments. In addition, the color measurement method might be partially responsible for the current finding. The color was measured using a reflectance spectrophotometer, which was designed to analyze the dentin and deep enamel color, ignoring reflection and surface irregularities.[18,24]

In addition to the L*, a*, b*, and ΔE values, the W index was also considered in alignment with previous studies.[27,28] There were no significant differences in the W index among the three groups at the end of the study. Although the ΔE values in group C were the greatest among the three groups immediately after in-office bleaching, a decrease was observed in the ΔE values, and an increase in tooth yellowness (increase in b* value) was found three weeks after the completion of in-office bleaching. A color recession was more evident when the regular dentifrice was used than when the whitening dentifrices were used. Therefore, whitening dentifrices could be considered suitable for maintenance following professional bleaching treatments. The whitening maintenance may benefit from the silica abrasive system in the whitening dentifrices.[16,28] In the literature, silica has strong abrasive properties, whereas sodium fluoride reduces enamel abrasion.[29,30] It is possible that this color alteration results from the gradual removal of extrinsic stains through use of the abrasive agents.

Based on the current findings, patients should be advised to refrain from using whitening dentifrices while undergoing professional tooth bleaching procedures. Clinicians should advise their patients to use whitening dentifrices after their bleaching treatments to better maintain the whitening results. However, further clinical studies would be beneficial to investigate the long-term effects of whitening dentifrices on bleached teeth.

CONCLUSIONS

Within the limitations of the present study, the following conclusions can be made:

1. The use of whitening dentifrice containing blue covarine during in-office bleaching procedures was associated with less color change compared with the conventional whitening and regular dentifrices.
2. The use of whitening dentifrices after in-office bleaching procedures produced a decrease in tooth yellowness, whereas regular dentifrice was associated with an increase in tooth yellowness.

Acknowledgements

The authors would like to acknowledge the Program for New Century Excellent Talents at Fujian Province University (grant number 2016B033); Joint Funds for the Innovation of Science and Technology, Fujian Province (grant number 2016Y9022); the Scientific Research Foundation for Returned Overseas Chinese Scholars, the State Education Ministry (grant number

2013B020); and the Science Foundation for Medical Innovation, Fujian Province (grant number 2015-CX-31).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Fujian Medical University. The approval code for this study is 20162309.

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.

(Accepted 29 March 2018)

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Effectiveness of Dental Bleaching With 37.5% and 6% Hydrogen Peroxide and Its Effect on Quality of Life

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Clinical Relevance

A gel with a low concentration of hydrogen peroxide (6%) achieves effective whitening with a low occurrence and intensity of sensitivity. It also generates a positive effect on psychosocial impact and esthetic self-perception among patients.

SUMMARY

Objective: This study investigated whether it is possible to achieve equally satisfactory results between 37.5% hydrogen peroxide (HP) gel and 6% HP gel. We also assessed the psychosocial impact and self-perception of esthetics generated by extracoronal tooth whitening.

Methods and Materials: A prospective, double-blind, randomized clinical trial was carried out. A total of 33 patients were selected from the clinic of the Faculty of Dentistry at the University of Chile. The patients included men and women over 18 years old without prior tooth whitening treatments, tooth decay, or restorations of the maxillary anterior teeth. The patients had tooth colors of A3 or less according to the Vita Classical scale, which was determined with a Vita Easy Shade spectrophotometer. The study was carried out with a "split-mouth" design. One side of each mouth was randomly treated with 37.5% HP, and the other side was bleached with 6% HP. Each group received 3 to 12 minutes of treatment with the respective gel applications. Two sessions of bleaching were carried out each week. A spectrophotometer was used to measure the total variation of color (ΔE), and a subjective evaluation was made with Vita Classical scale (ΔSGU) between the baseline (session 1) and different measurement times. We compared ΔE

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DOI: http://doi.org/10.2341/17-229-C

and ΔSGU for both agents using the Mann-Whitney test (α=0.05).

Results: In both groups, there was variation among the initial color and the color in the different measurement times. In the month after the treatment was completed, ΔE was 9.06 in the 37.5% HP group and 5.69 in the 6% HP group. The difference between the two groups was statistically significant starting in the second session (p=0.000).

Conclusion: There was a significant difference between the effectiveness of the bleaching gel concentrations of 37.5% and 6% HP according to spectrophotometer measurements and subjective evaluations. There was also a positive effect on psychosocial impact and esthetic self-perception among patients.

INTRODUCTION

Hydrogen peroxide (HP) is commonly used to treat the discoloration of teeth. It is a powerful oxidizer that separates the molecules of chromophores that remain on the teeth. The molecules become smaller molecules that reflect less light, which creates a whitening effect.[1] Evidence suggests that tooth whitening is safe and minimally invasive. However, some researchers believe that whitening could potentially lead to structural changes in the teeth tissues.[2]

The effects caused by whitening are controversial, especially for gels with high concentrations.[3] The effects and the diffusion of HP on hard tooth tissues depend on the concentration and contact time.[4] This has led some manufacturers to produce gels with lower concentrations of HP. The European Union banned the use of whitening agents at concentrations above 6%.[5] Despite this, patients continue to demand these treatments. Therefore, it is necessary to investigate bleaching agents to confirm their efficacy and safety under the new requirements.

Efforts are currently focused on achieving effectiveness with low concentrations of peroxide while minimizing the contact time between the tooth and bleaching gel to reduce the adverse effects.[6-8] This could be performed using low concentrations of dental whitening agents. However, it must be confirmed that the effectiveness does not differ significantly from that of the high concentrations that are usually used.[9,10] A recent study followed a cohort of patients treated with a low concentration HP gel. The study showed acceptable results and a low rebound of color at nine months and at one year.[6,8] However, the gel was catalyzed by light-emitting-diode or laser light. There are no reports on low concentrations of HP gels without light assistance that demonstrate the effectiveness of tooth bleaching.

There has been a large increase in the demand for improving cosmetic appearance, especially dental appearance. New reports link personality factors with choosing tooth whitening.[11,12] Thus, it is important to assess the possible changes in esthetic self-perception and the psychosocial impact among patients undergoing clinical tooth whitening using validated instruments. The effects of whitening can last for one year or more.[13] Thus, it seems relevant to consider whether possible psychosocial changes occur beyond the first few weeks after bleaching. Patients who undergo extracoronal tooth whitening experience a positive impact on psychosocial quality of life and self-perception.[6]

Therefore, the present study investigated whether it is possible to achieve equally satisfactory results between 37.5% HP gel and 6% HP gel in a split-mouth model. We also assessed the psychosocial impact and self-perception of esthetics generated by extracoronaral tooth whitening. The first null hypothesis was that there would be no difference in the effectiveness of the whitening gels at one month follow-up. The second null hypothesis was that the whitening would not affect the quality of life of patients.

METHODS AND MATERIALS

Study Design

This study was a double-blind, randomized, prospective clinical trial. The study was conducted according to the recommendations of CONSORT (Consolidated Standards of Reporting Trials),[14] as shown in Figure 1, as well as the principles of the Helsinki Convention.[15] The study was approved by the ethics local committee (approval number 15/001), and the trial was registered (NCT03217994).

Sample Description

Sample Selection—A total of 35 patients were selected from the clinic of the Faculty of Dentistry at the University of Chile. The patients had been seeking whitening treatment and volunteered to participate in the study. The selected patients had to meet all the inclusion criteria and signed informed consent forms adopted by the Ethics Committee of Faculty of Dentistry. The inclusion criteria were as follows:

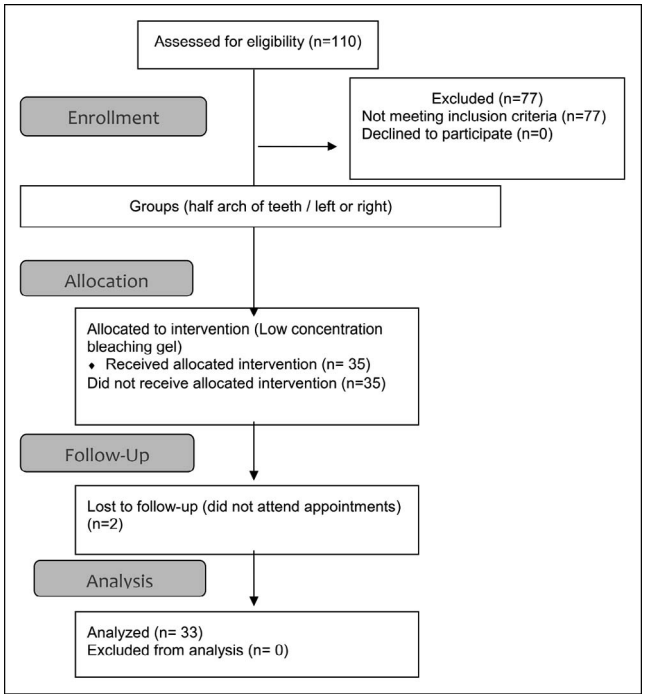

Figure 1. *Flow diagram of the clinical trial.*

- Age over 18 years (both sexes)
- Six present maxillary anterior teeth
- No caries
- No restorations (in the maxillary anterior teeth)
- No previous whitening treatments
- Tooth color value of A3 or darker (Vita Classical scale, Vita Zahnfabrik, Bad Säckingen, Germany), which was determined with a spectrophotometer (Vita Easy Shade Compact, Vita Zahnfabrik, Bad Säckingen, Germany) on the middle third of the vestibular surface of the maxillary central incisors

Patients were excluded based on the following criteria:

- Pregnant or nursing mothers
- In pharmacologic treatment
- Bruxism and patients who reported prior tooth sensitivity
- Previous tooth whitening (either at home or professionally)
- Visible dental cracks, developmental defects, or teeth stained by tetracycline or fluorosis in the maxillary anterior teeth
- Treatment with fixed appliances
- Periodontal disease or cancer
- Presence of noncarious cervical lesions or endodontics in the maxillary anterior teeth

Patients who experienced any pathologies that prevented them from entering the study (such as caries, periodontal disease, or dental sensitivity) were directed for treatment to the dental clinic of the Faculty of Dentistry of the University of Chile.

Sample Size—The sample size (n) was determined based on similar studies.[6] A minimum n of 25 patients per group was determined. A significance level of 5% was considered at (1-"β) 0.84 with a dropout rate of 5%, resulting in 35 patients.

Study Location—Treatments were carried out at the clinic of the Faculty of Dentistry of the University of Chile. During this period, volunteers were supervised by the researchers.

Procedure

Determination of Study Group—The study was carried out with a "split-mouth" design. Bleaching agents (HP) were randomly (pararell groups) calculated and assigned (SPSS 21, IBM, New York, NY, USA) to each hemiarch. The two operators were unaware of the product being used. To achieve this, auto-mix syringes from a Polaoffice+ whitening kit were used (SDI Limited, Bayswater, Australia). The syringes contained HP in the form of a thixotropic gel at a concentration determined by the manufacturer and marked properly. Each gel syringe was relabeled with a key number depending on the concentration of the agent, which was determined by an operator who was unaware of the procedures.

All color measurements were performed on the maxillary central incisors by different operators from those mentioned. In one group, the hemiarch (canine, lateral, and central incisors) was bleached with 37.5% HP (Polaoffice + 37.5% SDI Limited). In the other group, the hemiarch was bleached using 6% HP (Polaoffice + 6% SDI Limited).

Preliminary Phase—The procedures to be performed were verbally explained, and then each volunteer read and signed an informed consent form. In each case, a heavy silicone matrix (Speedex Putty, Coltene Whaledent, Altstätten, Switzerland) was prepared for both maxillary central incisors. These matrices were perforated at the height of the union of the cervical third and the middle third of the vestibular tooth face to standardize the color measurements with the spectrophotometer (Easyshade compact, Vita Zahnfabrik, Bad Säckingen, Germany). Another reason was to create a perfect fit with the nozzle of the spectrophotometer to help control the passage of light to the measurement site. The color of each maxillary central incisor was measured using

the spectrophotometer, which was previously calibrated according to the manufacturer's instructions.

Bleaching Protocol—Two whitening sessions were carried out with intervals of one week. At the beginning of each session, dental prophylaxis was done with a brush at low speed. Stone pumice and water were used on the maxillary anterior to remove the surface layer from the enamel so that it would not alter the effectiveness of the gels. We used a plastic lip separator and a light-cured blue resin gingival barrier (Gingival Barrier, SDI Limited) to protect the soft tissue. We homogeneously applied the different gels on the vestibular surfaces of each hemiarch. One hemiarch was treated with 37.5% HP, and the other was treated with 6% HP (Polaoffice+, SDI Limited).

The protocol included two sessions of treatment with three applications of 12 minutes of whitening gel each session (72 minutes of total contact). The gels were in full contact with the tooth surface and then were removed between each application with cotton rolls, which were moistened with water and dried carefully. At the end of the third application, we removed the gels, washed off all the excess with copious water, and removed the gingival barrier.

After Bleaching—All patients were instructed to avoid consuming foods with a high content of pigments, such as coffee, tea, wine, and beets during the study period.

Controls—At the end of the first session, we measured the tooth color with the calibrated spectrophotometer (Vita Easy Shade Compact), which has high reliability.[16] One week later, the same protocol was repeated. The time to control sessions (one week and one month post whitening) was considered.

Tabulation of Data—Data obtained in each period were tabulated according to the three axes of the CIELAB (CIEL*a*b* 1976 color space) system (L*, a*, and b*). We also calculated ΔE using the Pythagorean Theorem as follows:

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

The variation of each parameter at different times was always calculated in relation to the initial values (the color measurement prior to the first session of whitening).

Subjective Evaluation—For the subjective evaluation, we used the Vita Classical shade guide (Vita Classic, Vita Zahnfabrik), which ranges from lightest (B1) to darkest (C4) according to the color. Although the Vita Classical scale is not linear in the truest sense, we treated the changes as continuous with a linear ranking as in previous clinical trials of dental bleaching.[17] Two calibrated evaluators with a κ value of 0.85 recorded the shades of both central incisors at baseline, at each session, one week after treatment, and one month after. The perceptibility threshold considered was 2.7 ΔE units.[18] The color was registered over the middle third of the labial surface as established by the American Dental Association (ADA) guidelines.[19] The color difference was calculated as the number of shade guide units that the tooth changed toward the lighter end of the shade guide (ΔSGU). At the one-month control, the evaluation was done after dental prophylaxis and after waiting 15 minutes for rehydration of the teeth before color assessment.

Tooth Sensitivity Evaluation—The tooth sensitivity (TS) after whitening was assessed by the variables of occurrence and intensity. These data were obtained using a self-completed form as well as clinical evaluations during the sessions and immediately after by a visual analogue scale (VAS). For the VAS, we instructed the participants to place a line perpendicular to a 100-mm-long line, with a zero at one end indicating "no TS" and the other end indicating "unbearable TS."

Quality of Life

Before tooth whitening, as well as one week and one month after, all patients answered two questionnaires: (1) the Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ) and (2) the Oral Health Impact Profile for dental esthetics (OHIP-14). The questionnaires were answered under supervision of an examiner, who could answer the patients' questions.

PIDAQ[20] is used for psychometric assessment of the impact of psychosocial aspects of dental esthetics. It consists of 23 items rated on a five-point Likert scale (0 for total disagreement and 4 for full agreement). The items are divided into four subscales consisting of six questions on positive dental self-confidence, eight questions on three negative dimensions of psychological impact, three questions concerning esthetics, and eight questions on social impact. The total score is between 0 and 72 points. In addition, an analysis was done according to the subscales. A greater dental self-confidence subscale score indicated greater self-confidence. However, high scores on the subscales of psychological and social impact indicated adverse effects of esthetics.

Table 1: *Baseline Participant Characteristics*

Baseline Features	Groups (Hydrogren Peroxide Percentage)	
	37.5%	6%
Age (y; mean ± SD)	27.36 ± 9.28	
Minimum age (y)	20	
Maximum age (y)	53	
Male (%)	51.5	
L* (mean ± SD) ($p>0.05$)	85.85 ± 3.12	85.43 ± 3.21*
a* (mean ± SD)	0.22 ± 1.23	0.33 ± 1.50*
b* (mean ± SD)	28.06 ±3.39	28.64 ± 3.88*
Baseline Vita Classical SGU median (min:max)	9 (9:12)	9 (9:12)

Abbreviation: SD, standard deviation.
* $p>0.05$ *between groups.*

OHIP-14[21] is an instrument for evaluating esthetic self-perception. The questions are also answered on a five-point Likert scale indicating "very often" (4); "quite often" (3); "occasionally" (2); "almost never" (1); and "never or unknown" (0). A greater score indicated a worse patient self-perception of the cosmetic dentistry. To calculate the OHIP-14 score for each patient, the scores of the 14 questions were added to generate overall scores ranging between 0 and 56 points.

Statistical Analysis

The Shapiro-Wilk test was used to determine the normality of the dataset. The data distribution was not normal, so the Mann-Whitney test was used to compare the efficacy and sensitivity results in both groups. The Wilcoxon test was used to compare the variations between different measurement times. The statistical tests were performed using the software SPSS 21.0 (IBM, New York, NY, USA). Descriptive statistics of the scores on the scales of the PIDAQ and OHIP-14 esthetic surveys were determined, and the results were compared for different evaluation time points using the Wilcoxon test. Besides the selected demographic variables such as age and sex, the data were coded and treated anonymously.

RESULTS

Description of the Sample

This study evaluated 110 patients who voluntarily came to the local Faculty of Dentistry clinic. Using a "split-mouth" design, each group (37.5% and 6% HP) comprised n=35 patients (selected according to the inclusion criteria). However, two patients left the study due to noncompliance with the appointments, so only 33 patients were considered in the analysis (Figure 1). The average age of the sample was 24 years old, with a range of 20 to 47 years. Their baseline features are presented in Table 1.

Effectiveness of Whitening

Objective Evaluation—Both groups achieved effectiveness ($\Delta E > 5$) with differences of more than 3 ΔE units at one month after bleaching ($p<0.001$; Table 2).

Subjective Assessment—Both groups had a whitening effectiveness of more than 5 SGU units at one month after whitening, but there was a statistically significant difference between groups ($p<0.05$; Table 3).

Sensitivity—Only four patients noticed sensitivity, and the mean sensitivities after the first session according to VAS were 0.48 ± 1.20 in the 37.5% group and 0.41 ± 1.31 in the 6% group. However, differences between groups were not statistically significant ($p=0.531$). The mean sensitivity after the second session was 0.41 ± 1.20 in the 37.5% group and 0.35 ± 1.37 in the 6% group, but again the differences were not statistically significant ($p=0.450$).

Quality of Life

Psychosocial Impact (PIDAQ)—At one week and one month, there were statistically significant differences from the baseline values of PIDAQ, which indicate a positive impact on the factors (Table 4).

Esthetic Self-Perception (OHIP)—All factors except functional limitation had significantly lower

Table 2: *Comparison of ΔE Values at Different Times*

Assessment Points	Color Change by ΔE		
	37.5% Hydrogen Peroxide	6% Hydrogen Peroxide	Mann-Whitney Test
Baseline vs one week during bleaching	3.79 ± 4.43	3.53 ± 3.04	0.493
Baseline vs two weeks during bleaching	6.95 ± 3.33	4.37 ± 2.34	0.001
Baseline vs one week after bleaching	8.94 ± 3.07	6.34 ± 3.82	0.001
Baseline vs one month after bleaching	9.06 ± 2.96	5.69 ± 3.06	0.000

Table 3: *Comparison of ΔSGU Values at Different Times*

Assessment Points	Color Change by ΔE		
	37.5% Hydrogen Peroxide	6% Hydrogen Peroxide	Mann-Whitney Test
Baseline vs one-week bleaching	3.90 ± 1.90	3.52 ± 1.90	0.345
Baseline vs before two-week bleaching	4.77 ± 2.10	4.29 ± 2.10	0.325
Baseline vs two-week bleaching	6.68 ± 1.60	6.26 ± 1.60	0.196
Baseline vs one week after bleaching	6.68 ± 1.60	6.10 ± 1.7	0.106
Baseline vs one month after bleaching	6.71 ± 1.80	5.87 ± 1.8	0.019

values compared with the baseline. At one month, all factors measured had a statistically significant difference compared with the baseline. In the overall score, the difference was also significant at one week and one month (Table 5).

DISCUSSION

This study compared the effectiveness of tooth whitening with gels containing 6% and 37.5% HP without light treatment. Tooth color was measured up to the one month after whitening using a spectrophotometer and a subjective assessment. Based on the results, we rejected the null hypothesis that bleaching with 6% HP was as effective as that with 37.5% HP.

Prior to the intervention, the spectrophotometer measurements showed that there was no significant difference between the initial parameters of the two groups (Table 1) on any of the three axes of the color system. This is an advantage of the split-mouth design, because there is greater variability between the characteristics of each hemiarch in the same patient.[7,22,23] Bleaching was considered effective in both groups because they presented a ΔE of at least five units during the control month (G [6%]: ΔE = 5.69). In the 37.5% group, the highest value of ΔE occurred in the monthly control (ΔE = 9.06).

Table 4: *PIDAQ Results at Different Time Points*

Dimension	Time Points		
	Baseline	1 Week After Bleaching	1 Month After Bleaching
Dental self-confidence	18 (10:63)	23 (15:68)[a]	23 (16:30)[a]
Social impact	17 (9:34)	16 (8:34)[a]	13 (8:29)[a]
Psychological impact	19 (8:28)	15 (6:26)[a]	13 (6:23)[ab]
Esthetic concern	7 (3:15)	6 (3:10)[a]	5 (3:10)[a]
Sum	60(44:86)	59 (38:92)	55 (36:75)[ab]

[a] *Statistically significant difference (Wilcoxon test, p<0.05) versus baseline.*
[b] *Statistically significant difference (Wilcoxon test, p<0.05) versus 1 week after bleaching.*

From the measurements at the start of the second session, we began to see a separation between the values of each group, with a difference of more than two units in ΔE that increased over time (Table 2). Despite the differences between the final values, only one patient noticed a difference between both hemiarches (was retreated). Prior to bleaching, we told the patients that we would work to match the colors of the different hemiarches if they were unhappy with the esthetic results. This probably occurred because the difference between the colors of both groups was not significant enough due to the thresholds of each person.

Related to the objective effectiveness, dental in-office whitening systems can be categorized into groups of high or low concentration of HP (>35% or <20%, respectively). Highly concentrated groups traditionally show a high effectiveness, with more than eight units of ΔE in color change, in treatments of around 100 minutes of segmented contact in different sessions using different protocols.[24,25] In contrast, low concentrated agents traditionally have been less effective, with ΔE of around five units.[10,26,27] In our study we obtained color changes

Table 5: *Effect of Bleaching in the Esthetic Self-perception Evaluated With the OHIP Questionnaire*

Dimension	Time Points		
	Baseline	1 Week After Bleaching	1 Month After Bleaching
Functional limitation	3 (0:7)	3 (0:6)	2 (0:6)[a]
Physical pain	3 (0:7)	2 (0:4)[a]	2 (0:6)[a]
Psychological discomfort	4 (0:7)	3 (0:6)[a]	3 (0:5)[a]
Physical disability	1 (0:6)	1 (0:4)[a]	0.5 (0:2)[a]
Psychological disability	1 (0:5)	0.5 (0:3)[a]	0 (0:3)[a]
Social disability	0 (0:4)	0 (0:3)[a]	0 (0:2)[a]
Handicap	0 (0:4)	0 (0:3)[a]	0 (0:3)[a]
Sum	14 (6:33)	11 (3:21)[a]	10 (0:19)[ab]

[a] *Statistically significant difference (Wilcoxon test, p<0.05) versus baseline.*
[b] *Statistically significant difference (Wilcoxon test, p<0.05) versus 1 week after bleaching.*

around six units of ΔE with a low concentrated gel. However, this effectiveness is similar to low concentration gels assisted by a LED/laser light. The gel used in this study comes with the alkaline components separated in two compartments that are self-mixed at the time of application, reducing the possibility of inactivation of the HP, perhaps representing an advantage in the effectiveness of the product compared with other forms of presentation.[24]

Regarding the variations in ΔE, the results of this study are in accordance with a previous study by Martin and others,[22] who also compared the effectiveness of 6% with 37.5% hydrogen peroxide. However, they modified both agents with nitrogen titanium dioxide and activation by LED light. However, Martin and others obtained one difference in that the values of ΔE were lower in both groups in the monthly control (2.41 units). Their 37.5% group obtained lower values than our 37.5% group, and their 6% group obtained better performance than our 6% group.

In addition, although there was a statistically significant difference between the two groups according to the objective spectrophotometer measurements, the difference in ΔE was less than four units and did not show a significant difference from subjective assessments using color tablets. Furthermore, no patient was unhappy with the results based on the subjective assessments. Notably, in this clinical study, the 6% gel did not require light assistance as in the trial published by Martin and others.[22] This reduces the cost of the treatment and is advantageous for the clinician.

In vitro studies have looked at reducing the concentration of the bleaching agent. There is a significant decrease in the penetration of HP and its byproducts through the mineralized tissues of the tooth to reach the pulp.[29] Consequently, the effects on the pulp tissue can be reduced, and tooth sensitivity can be avoided. This is why efforts have focused on demonstrating the effectiveness of new bleaching protocols and agents with lower concentrations.[10] Surely, future recommendations will involve the use of low concentrations for tooth whitening.

One of the strengths of this research in comparison with previous studies is the split-mouth design, which reduces the variability between the groups studied by using every patient as their own control.[23] However, there was no previous certainty that the results of both products would be similar.

The intensity and occurrence of sensitivity were low, which could be related to the use of both gels with a more neutral pH. This coincides with the results of a recent clinical trial that used the same gel with a concentration of 37.5%.[24] That study reported lower sensitivity than when using gels with a more acidic pH. Interestingly, there was no difference between the reported sensitivity between the two gels of different concentrations, which could be due to the strict selection of patients without previous history of sensitivity.

In general, traditional highly concentrated HP gels generate higher values of sensitivity in intensity and duration.[25] The literature reports prevalence of patients with sensitivity induced by dental whitening between 45% and 90% with moderate intensity and in some cases high.[30] However, the new lower concentrations of HP (15% or 6%) definitely report very low sensitivity both in prevalence and intensity.[7,9,10] Clearly the most common adverse effect of tooth whitening is being controlled by the emergence of new whitening gel technologies.

The second hypothesis was rejected because we noticed a positive effect on the psychosocial impact and on the esthetic self-perception of the patients. The study results showed that there were significant changes in the values of the PIDAQ and OHIP-14 questionnaires after comparing the scores prior to clinical whitening with those obtained after the procedure. This difference in values showed that there was a change in patients' psychosocial aspects. Thus, by improving the dental esthetics through whitening, there was a positive change in the psychosocial impact and self-perception of these patients. There were no significant differences between the post-treatment measurements. Therefore, it can be concluded that tooth whitening is a modifier of psychosocial aspects and the self-perception of esthetic dental factors. Once the procedure was over, there were no other esthetic changes.

Dental self-reliance measures the influence of esthetic dentistry on the self-image of an individual. The appearance of the mouth and smile play important roles in the assessment of facial attractiveness, which undoubtedly contributes to improving self-esteem.[31] The results of this study suggest that extracoronal tooth whitening produces an increase in dental self-confidence, which remains over time. This finding shows that this factor is associated with more favorable attitudes toward oral health and a higher degree of satisfaction with respect to better self-image.[32]

The measurement of social impact is aimed at assessing potential problems that an individual may face in social situations due to a subjectively unfavorable dental appearance. The third dimension of psychological impact evaluates an individual's feelings of inferiority or unhappiness in comparison to others. The fourth dimension concerns esthetics and includes data related to the concern or disapproval that an individual's dental appearance generates when they look at the mirror, photographs, or videos.[20] In terms of these three negative dimensions (social impact, psychological impact, and esthetic concerns), the results obtained show a decrease in scores when comparing data from the beginning of the treatment versus the assessments at one week and one month after whitening. Therefore, extracoronal tooth whitening generates an immediate and short-term positive psychosocial impact.

In the total scores obtained from the OHIP-14 questionnaire, there was a statistically significant decrease compared with the evaluation prior to whitening. This indicates that extracoronal whitening produces a substantial improvement in the self-perception of cosmetic dentistry in patients and a noticeable decrease in the dimensions of physical, psychological, and social disabilities, as well as physical pain and handicaps. These values decreased significantly with the treatment and show important implications in biopsychosocial health approaches, because the disadvantages experienced due to cosmetic dental problems may profoundly affect a person's self-esteem, interactions, adaptations to their environment, personal relationships, job opportunities, and fundamental aspects that affect quality of life.[32,33]

Regarding the dimension of functional limitation, the analyses showed a positive effect at just one month after treatment. No changes occurred after one week. This finding demonstrates that the effect is not immediate, and the patient requires interaction with their environment and a chance to build interpersonal relationships to accomodate this positive change.[34] This positive effect was maintained for at least three months. The dimension that had the greatest improvement was psychological discomfort. The improvement was observed at one week after completing treatment and remained constant during all subsequent evaluations. These improvements are consistent with the results obtained in a study from 2015, in which there were also improvements in the different dimensions of OHIP-14.[22]

Comparison processes play an important role in psychosocial well-being and feelings of inferiority to others, and they could result in dysphoric states.[35] This study showed that there is an increase in psychological well-being after tooth whitening, which remained steady over time. Whitening improves a patient's own self-satisfaction, and they feel better and safer when they have teeth with a color that pleases them. The PIDAQ and OHIP-14 tools demonstrated that there are positive changes in both the psychosocial well-being of patients and the self-perception of cosmetic dentistry at the end of the whitening period. These changes also remained at one and three months after treatment, which corroborates the hypothesis that the psychosocial impact and esthetic self-perceptions are positively changed by extracoronal tooth whitening.

It would be useful in future research to include comparative studies contrasting color changes after extracoronal tooth whitening (either through spectrophotometer or shadeguides) versus the changes in psychosocial aspects of cosmetic dentistry patients in the medium and long term. It would also be desirable to compare the psychosocial changes of patients in relation to the different techniques of extracoronal bleaching (in-office vs at home) and with different concentrations. The esthetic self-perception and psychosocial impact could also be compared among patients undergoing tooth whitening vs untreated patients. This would shed more light on whether the positive changes are due to bleaching and not other factors. Finally, future studies could use other questionnaires to measure whether there are improvements in the quality of life of a patient after undergoing tooth whitening. Although this study shows significant differences in quality of life measurements, it is recommended to carry out studies with controls of parallel groups of patients that are not subjected to tooth whitening to test the controlled effect on the quality of life.

CONCLUSIONS

Whitening with both 37.5% HP and 6% HP is effective according to measurements with a spectrophotometer. There was a statistically significant difference between the effectiveness of bleaching between concentrations starting from the second session of treatment.

There is a positive psychosocial impact in patients undergoing extracoronal tooth whitening when comparing the baseline measurements with those taken one week and one month after treatment. There is an increase of self-confidence and psychological well-being compared with the start of the

whitening with the measurements at one week and one month after treatment.

Acknowledgements

The author dedicates this article to his two children, Elisa and Eduardo due to their enormous inspiration. Class of Dentistry 1994 - Universidad de Chile. Fondecyt 1170575

Regulatory Statement

This study was conducted in accordance with all the provisions of the approval of the Local Ethics Committee guidelines and policies of the Comité de Etica Fouch. The approval code for this study is 15/001.

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.

(Accepted 4 February 2018)

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Laboratory Research

Surface Roughness of Ceramic-Resin Composites After Femtosecond Laser Irradiation, Sandblasting or Acid Etching and Their Bond Strength With and Without Silanization to a Resin Cement

Z Demirtag • AK Culhaoglu

Clinical Relevance

The femtosecond laser irradiation and silanization seem to be a promising treatment for improving the bond strength of resin cement to Vita Enamic and Lava Ultimate. It is expected that dental configurations of ultra short pulse lasers such as femtosecond lasers will reduce the drawbacks of available dental lasers used for roughening restorative materials.

SUMMARY

Objectives: The aim of this study was to investigate the effects of femtosecond laser irradiation, sandblasting, or acid etching treatments on the surface roughness of ceramic-resin composites and also shear bond strength (SBS) with and without silanization to a resin cement.

Methods: Samples of Vita Enamic (VE; Vita Zahnfabrik, Bad Säckingen, Germany) and Lava Ultimate (LU; 3M ESPE, Seefeld, Germany) were classified into control (no treatment), sandblasting, hydrofluoric acid, and femtosecond laser groups (n=30). Surface roughness was determined using two-dimensional contact profilometry. Surface topography was evaluated using a three-dimensional contact profilometer and a scanning electron microscope. Then groups were divided into two subgroups with similar surface roughness values, including control (C), control + silane (C-S), sandblasting (SB), sandblasting + silane (SB-S), hydrofluoric acid (HF), hydrofluoric acid + silane (HF-S), femtosecond laser (FS), and femtosecond laser + silane (FS-S) groups (n=15). Panavia F 2.0 resin cement was applied

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DOI: http://doi.org/10.2341/17-391-L

to the sample surfaces using an SDI SBS rig (SDI Limited, Bayswater, Australia). The SBS test was performed after water storage (24 h, 37°C) and thermocycles (2000 cycles, 5°C to 55°C), and failure modes were evaluated.

Results: The highest surface roughness was observed in the FS group, and the highest SBS was observed in the FS-S group for both VE and LU ($p<0.001$). Silanization improved the SBS of VE significantly ($p<0.001$) in all surface treatments but did not improve that of LU except in the FS group ($p=0.004$). There was a significantly moderate negative correlation in the VE/SB group ($p=0.012$) and a moderate positive correlation in the VE/HF group ($p=0.049$).

Conclusions: Femtosecond laser irradiation was found to be more effective than sandblasting or acid etching in increasing the surface roughness, and it was also the most effective surface treatment with silanization on the SBS of a resin cement to the ceramic-resin composites.

INTRODUCTION

Ceramics and composite resins are leading restorative materials that can be used with computer-aided design/computer-aided manufacturing (CAD/CAM) systems. Despite their superior mechanical and esthetic characteristics, ceramics have disadvantages, such as antagonist tooth wear and brittleness. On the other hand, composite materials do not have these disadvantages but cause polymer shrinkage and possess relatively weak mechanical properties.[1] Manufacturers have been improving new concept restorative materials combining the advantageous properties of ceramics and composites.[2] Thus, these materials, which could show similar mechanical properties to dentin or enamel, have contributed to the manufacturing of biomimetic materials.[3,4]

Lava Ultimate (LU) is a nanoceramic resin containing nanoceramic particles embedded in a highly cross-linked resin matrix. LU contains (by weight) 80% nanoceramic particles and 20% resin matrix,[5] and its composition was based on the Filtek Supreme Ultra (3M ESPE, Seefeld, Germany) resin-based composite.[6] It is different from resin-based composites in that it uses a high-temperature polymerization process for its manufacturing,[7] presenting more advanced physical, mechanical, and optical properties than the conventional manufacturing process of resin-based composites.[8-11] Vita Enamic (VE) is a hybrid material with a dual-network structure manufactured by infiltrating polymer into a porous feldspar ceramic enriched with aluminum oxide. VE is (by weight) 86% ceramic and 14% polymer.[12] Both these materials combine the positive characteristics of ceramic and composite materials because of their dual-network structure, and they are classified as ceramic-resin composites (CRC).[13,14]

Micromechanical retention, stability, and wettability are indispensable to facilitating a durable and reliable bond between the adhesive cement and restorative material.[15,16] Surface treatments are performed to enhance surface energy between the cement and the restorative material by increasing the surface roughness. Hydrofluoric acid etching selectively removes the glass matrix within the ceramic base and exposes crystal particles, thereby creating microporosities on the surface.[17] Sandblasting with aluminum oxide (Al_2O_3) both cleans the material surface and increases the surface area.[15] However, long sandblasting times can lead to cracks and volume loss.[18] Laser irradiation increases surface roughness and mechanical retention by melting the material's surface.[19] Silanization facilitates chemical bonding of two different organic and inorganic materials on ceramic surfaces.[20] Sandblasting and/or acid etching are commonly used methods to enhance micromechanical bonding, and silanization is a commonly used method to enhance chemical bonding.[21] These methods can be combined to enhance both micromechanical and chemical bonding.[18,22]

Studies using various laser irradiation applications to modify dental ceramics[19,23] and indirect composites[24,25] have reported promising results; however, these studies have some limitations. Yucel and others[26] and Ersu and others[27] observed crack formation in ceramics associated with thermal damage using Nd:YAG laser and CO_2 laser, respectively. However, Moezizadeh and others[28] reported that Er,Cr:YSGG laser may reduce bonding by increasing thermal destruction in indirect composites. Such disadvantages of long-pulsed lasers brought ultrashort-pulsed femtosecond (FS) lasers to the forefront. As FS lasers have a short interaction time, they limit temperature distribution in the dental tissue and reduce energy loss on the surface. This feature not only provides a much higher surface energy than other laser systems but also causes minimal thermal and mechanical damage.[29] FS lasers can create repeated microvoids in different

Table 1: *Brand Names, Manufacturers, Compositions, and Lot Numbers of Materials Used in the Present Study*

Material	Brand Name	Manufacturer	Composition	Lot Numbers
Hybrid ceramic	Vita Enamic	Vita Zahnfabrik (Bad Säckingen, Germany)	86 wt% feldspar ceramic, 14 wt% polymer	51100
Resin nanoceramic	Lava Ultimate	3M ESPE (Seefeld, Germany)	80 wt% nanoceramic, 20 wt% resin	N606702
Dual-cure resin cement	Panavia F 2.0	Kuraray Medical Inc (Okayama, Japan)	Paste A: MDP, DMA, silanated silica, camphorquinone, catalysts, photoinitiator Paste B: DMA, silanated barium glass, sodium fluoride, catalysts, accelerator	990076 980014
Silane	Clearfil Ceramic Primer	Kuraray Medical Inc	3-MPS, MDP, ethanol	940008
Hydrofluoric acid gel	Vita Ceramics Etch	Vita Zahnfabrik	5% hydrofluoric acid	37160
Al_2O_3 powder	Korox 50	BEGO (Bremen, Germany)	99.6% Al_2O_3 (50μm)	14 361781112

Abbreviations: MDP, 10-methacryloxydecyldihydrogenphosphate; DMA, dimethacrylate; MPS, methacryloxypropyltrimethoxysilane; Al_2O_3, aluminum oxide.

shapes and depths by using software. FS laser irradiation has become more widely used because this procedure provides sensitive and controlled roughening without altering the surface characteristics of the material.[29-32]

To date, only a few studies have examined surface treatments for CRC materials and their bond strength with resin cement. The aim of this study is to evaluate the effects of various surface treatments on the surface roughness and bond strength of resin cement to CRC CAD/CAM blocks. The null hypotheses (H_0) of this study are as follows: 1) femtosecond laser irradiation would not enhance surface roughness more than aluminum oxide sandblasting and hydrofluoric acid etching, 2) femtosecond laser irradiation would not enhance the bond strength to resin cement more than aluminum oxide sandblasting and hydrofluoric acid etching, and 3) silanization would not enhance the bond strength to resin cement.

METHODS AND MATERIALS

Experimental Design

All the materials used in the present study are listed in Table 1. Two hundred and forty-eight samples (5×5×2 mm) were prepared from VE and LU CAD/CAM blocks using a low-speed cutting saw (Micracut 201, Metkon, Bursa, Turkey). The samples were polished under water using 240-, 800-, and 1200-grit silicon carbide abrasive papers (Minitech 233, Presi, Grenoble, France) to obtain smooth and standard sample surfaces. After polishing, all samples were cleaned by keeping them for five minutes in an ultrasonic cleaner (Eurosonic Energy, Euronda, Vicenza, Italy) containing distilled water. The VE and LU material groups (n=124) were first divided into the following four groups (n=31): control, sandblasting, hydrofluoric acid, and femtosecond laser groups. The relevant surface treatment methods were used for each group. After measuring surface roughness, the sample with a roughness value closest to the average value was selected from each group for surface analysis. Then each group (n=30) was divided into two subgroups with similar average roughness values. At this stage, silane was applied to one of the groups, whereas no additional procedure was performed in the other groups. Thus, the following eight surface treatment groups were formed: control (C), silane (C-S), sandblasting (SB), sandblasting + silane (SB-S), hydrofluoric acid (HF), hydrofluoric acid + silane (HF-S), femtosecond laser (FS), and femtosecond laser + silane (FS-S).

- Group C: No surface treatment was applied.
- Group C-S: Silane coupling agent (Clearfil Ceramic Primer, Kuraray Medical Inc, Okayama, Japan) was applied to the surface for five minutes.
- Group SB: The sample surfaces were sandblasted with 50 μm of Al_2O_3 at 2 bar for five seconds at a distance of 10 mm (BEGO Easyblast, Bremen, Germany). A specifically designed holder was used to standardize the distance between the sample surface and the nozzle. After sandblasting, the samples were again ultrasonically cleaned in distilled water for five minutes.

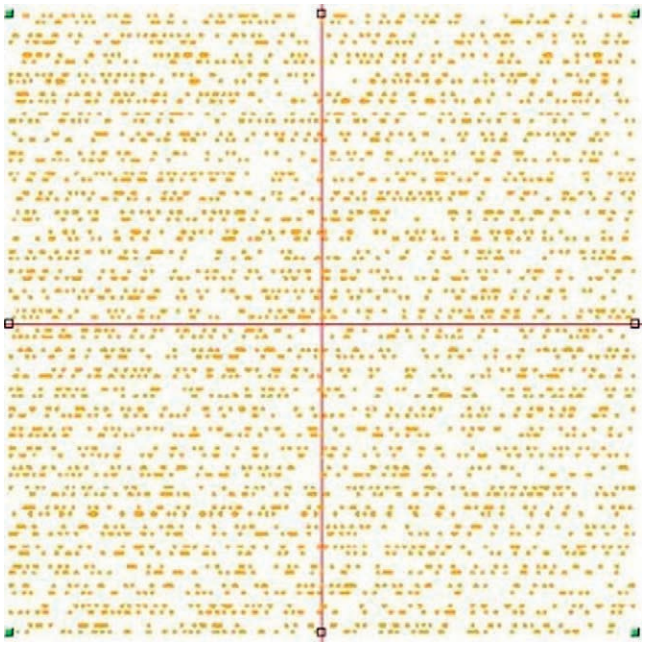

Figure 1. *The standard roughening pattern used by femtosecond laser.*

- Group SB-S: Silane was applied after sandblasting.
- Group HF: The sample surfaces were etched with 5% HF (Vita Ceramic Etch, Vita, Bad Säckingen, Germany) for 60 seconds, rinsed with water for 20 seconds, and then dried using oil-free airflow.
- Group HF-S: Silane was applied after etching with HF acid.
- Group FS: FS laser (Integra-C-3.5, Quantronix, New York, NY, USA) was used with an output power of 300 mW per pulse, an 800-nm wavelength, a 90-femtosecond pulse duration, a 1-kHz repetition frequency, and an energy density of 10.62 J/cm^2. Then laser pulses with a diameter of approximately 60 μm were focused on the sample surfaces; this was done by passing the beams through an f-theta lens (Q-Mark, Quantronix) with a focal length of 11 cm. The samples were roughened using a standard roughening pattern (Figure 1).
- Group FS-S: Silane was applied after irradiating with FS laser.

Surface Roughness Analysis

The average roughness values (R_a) of the samples were determined using a two-dimensional contact profilometer (Perthometer M2, Mahr, Göttingen, Germany) with a measurement length of 1.75 mm and a cutoff value of 0.25 mm. The roughness value (μm) was calculated by taking the average of the values obtained from five different regions of the samples. The profilometer was calibrated using a reference block after every 10 measurements.

Surface Topography Analysis

The surface topography of the samples was evaluated using a three-dimensional (3D) contact profilometer (Nanomap500LS, AEP Technology, Saratoga, CA, USA). The vertical dynamic range was set to 500 μm, the scan range was set to 2 × 2 mm, and the stylus loading force was set to 12 mg. The obtained images were interpreted using a color scale and graphics. Each color in the images represents a different value; the negative values represent pits, and the positive values represent peaks.

Scanning Electron Microscopic Analysis

Two-dimensional surface morphology of the samples was analyzed using a scanning electron microscope (SEM; JSM-5600 LV, JEOL, Tokyo, Japan). Each sample was coated with gold-palladium, and the roughness images of the samples were recorded 1000× magnification except FS samples (250×).

Bonding Procedure

An SDI SBS rig (SDI Limited, Bayswater, Australia) was used to prevent overflow of the cement from the interface and to provide a standard bonding area. Panavia F 2.0 resin cement (Kuraray, Osaka, Japan), which was mixed in accordance with the manufacturer's instructions, was layered in four steps into the rigs that were fixed onto the sample surface. Each layer was polymerized using Elipar S10 (3M ESPE, Seefeld, Germany) with a light intensity of 1200 mW/cm^2 for 20 seconds. After the polymerization of the final layer, oxygen inhibition gel was applied onto the cement layer for 10 minutes. The samples that were stored in 37°C distilled water were thermocycled for 2000 cycles in water between 5°C and 55°C with a dwell time of 30 seconds and transfer time of five seconds.

Shear Bond Strength Test

The shear bond strength (SBS) of a CRC to resin cement was tested with a knife-edge chisel by using a universal testing machine (Shimadzu AGS-X, Shimadzu Corp, Tokyo, Japan) at a crosshead speed of 0.5 mm/min until fracture. The maximum stress of the CRC-resin cement was calculated by the following formula: Stress (MPa) = Load (N)/Area (mm^2). The standard bond area (9.62 mm^2) was obtained using the SDI rig stainless-steel mold, which has an internal diameter of 3.5 mm.

Table 2: *Mean (Standard Deviation) of the Average Surface Roughness (Ra/μm) of Ceramic-Resin Composites According to Different Surface Treatments*[a]

Surface Treatments	Vita Enamic	Lava Ultimate
C	0.07 (0.02) Aa	0.05 (0.02) Ba
SB	1.77 (0.15) Ab	2.00 (0.19) Bb
HF	0.65 (0.05) Ac	0.15 (0.02) Bc
FS	6.88 (0.71) Ad	6.65 (0.83) Ad

Abbreviations: C, control; SB, sandblasting; HF, hydrofluoric acid; FS, femtosecond laser.
[a] *Same uppercase letters in each row indicate no significant difference, while same lowercase letters in each column indicate no significant difference according to the* post hoc *Tamhane test (*$p>0.05$*).*

Failure Mode Analysis

Failed surfaces were examined using a stereomicroscope (NZ.1902-P, Euromex, Arnhem, Netherlands) at 40× magnification. Failure modes were classified as adhesive (between cement and ceramic), cohesive (within CRC), and mixed (simultaneous adhesive and cohesive failure).

Statistical Analysis

The data were analyzed using the statistical software (IBM SPSS Statistics 20, SPSS Inc and IBM Corp, New York, NY, USA) at a significance level of 0.05. The normality of the data was determined using the Kolmogorov-Smirnov test (normal, $p>0.05$). The surface roughness values were analyzed using one-way analysis of variance and the *post hoc* Tamhane test. The SBS values were analyzed using the Kruskal-Wallis test and the Mann-Whitney U-test. The relationship between the surface roughness and SBS values was analyzed using Spearman's correlation test.

RESULTS

Surface Roughness Analysis

There was a significant difference between subgroups of VE and subgroups of LU (both $p<0.001$). The FS group showed the highest values for VE and LU (R_a, 6.88±0.71 μm; R_a, 6.65±0.83 μm, respectively), followed by the SB, HF, and C groups in descending order. However, the VE/C (R_a, 0.07±0.02 μm) and VE/HF (R_a, 0.65±0.05 μm) groups showed significantly higher roughness values than the LU/C (R_a, 0.05±0.02 μm) and LU/HF (R_a, 0.15±0.02 μm) groups, and the LU/SB (R_a, 2.00±0.19 μm) group showed significantly higher values than the VE/SB (R_a, 1.77±0.15 μm) group (all $p<0.001$) The surface roughness results are displayed in Table 2.

Surface Topography Analysis

While the LU/C group showed a higher roughness value, the VE/C and LU/C groups showed a smooth surface structure (Figure 2A,E). While the LU/SB group showed higher roughness, the VE/SB and LU/SB groups had rough surfaces with irregular peaks and valleys (Figure 2B,F). While the VE/HF group showed a very indented surface topography, the LU/HF group showed a less indented and rougher surface topography (Figure 2C,G). The VE/FS and LU/FS groups, on the other hand, showed similar surface topographies with deep parallel pits (Figure 2D,H).

SEM Analysis

Although microstructural changes were observed in the images, no defects or microcracks were observed. A smooth surface with irregular micropores was observed in the VE/C group; the polymer phase was dark gray, and the ceramic phase was light gray (Figure 3A). In the LU/C group, on the other hand, a more smooth and homogenous surface with smaller micropores was observed (Figure 3E). Prominent peaks and pits with crevices were observed in the VE/SB group (Figure 3B). In the LU/SB group, however, there were less prominent peaks and pits (Figure 3F). In the VE/HF group, it was observed that the ceramic phase was partially resolved and there were many micropores, which is suitable for micromechanical bonding (Figure 3C). In the LU/HF group, however, shallower micropores were observed (Figure 3G). The VE/FS and LU/FS groups showed deep pits that were distributed in accordance with the standard roughening pattern, whereas finer-grained particles were observed in the LU/FS group (Figure 3D,H).

SBS Analysis

There was a significant difference between the groups in both materials (both $p<0.001$). The VE/SB-S (18.64 MPa), VE/HF-S (22.99 MPa), and VE/FS-S (25.55 MPa) groups showed significantly higher SBS than the VE/SB (10.47 MPa), VE/HF (9.86 MPa), and VE/FS (12.45 MPa) groups, respectively (all $p<0.001$). However, the VE/FS-S and VE/HF-S groups showed significantly higher SBS than the VE/SB-S group ($p=0.012$ and $p=0.031$, respectively), and the VE/SB-S group showed significantly higher SBS than the VE/SB, VE/HF, and VE/FS groups (all $p<0.001$).

The LU/FS-S (18.14 MPa) group showed significantly higher SBS than the LU/FS (11.59 MPa) group ($p=0.004$). However, the LU/FS-S group

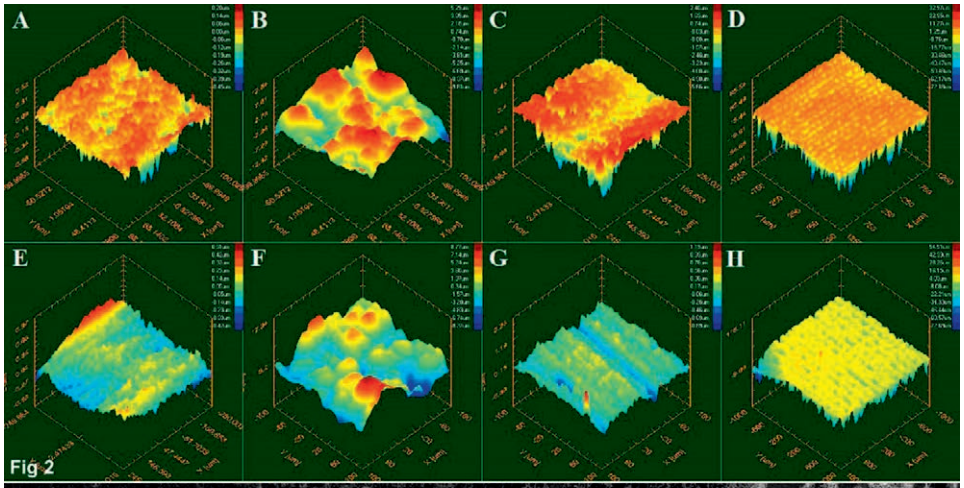

Figure 2. *3D profilometer images. (A): Vita Enamic/Control-VE/C. (B): Vita Enamic/Sandblasting-VE/SB. (C): Vita Enamic/Hydrofluoric Acid-VE/HF. (D): Vita Enamic/Femtosecond Laser-VE/FS. (E): Lava Ultimate/Control-LU/C. (F): Lava Ultimate/Sandblasting-LU/SB. (G): Lava Ultimate/Hydrofluoric Acid-LU/HF. (H): Lava Ultimate/Femtosecond Laser-LU/FS.*

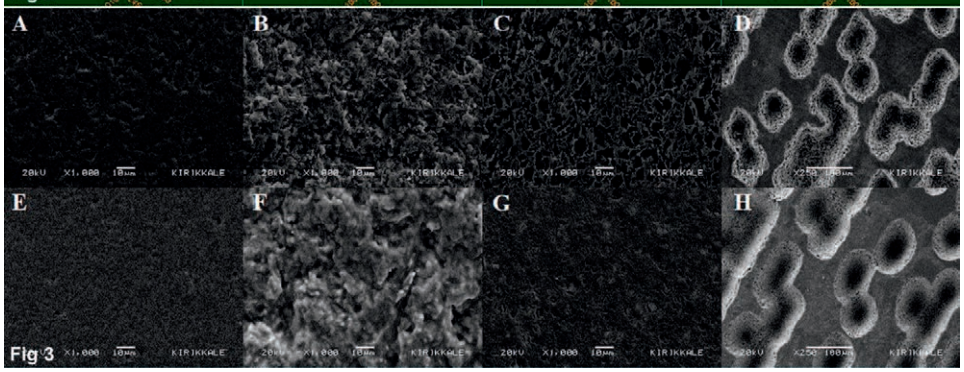

Figure 3. *SEM images. (A): Vita Enamic/Control-VE/C. (B): Vita Enamic/Sandblasting-VE/SB. (C): Vita Enamic/Hydrofluoric Acid-VE/HF. (D): Vita Enamic/Femtosecond Laser-VE/FS. (E): Lava Ultimate/Control-LU/C. (F): Lava Ultimate/Sandblasting-LU/SB. (G): Lava Ultimate/Hydrofluoric Acid-LU/HF. (H): Lava Ultimate/Femtosecond Laser-LU/FS.*

showed significantly higher SBS than the LU/HF (11.33 MPa) and LU/HF-S (12.20 MPa) groups (p=0.004 and p=0.017, respectively), whereas these groups showed significantly higher SBS than the LU/SB (6.02 MPa) and LU/SB-S (6.32 MPa) groups (all p<0.001).

The VE/SB, VE/SB-S, VE/HF-S, and VE/FS-S groups showed significantly higher SBS than the LU/SB, LU/SB-S, LU/HF-S, and LU/FS-S groups (p=0.002, p<0.001, p<0.001, and p=0.005, respectively). The SBS results are displayed in Table 3.

Failure Mode Analysis

The failure modes observed in the VE groups were as follows: completely adhesive failures in the VE/C group, mostly adhesive in the VE/C-S (93%) and VE/SB (60%) groups, mostly cohesive in the VE/HF-S (60%) and VE/FS-S (53%) groups, and mostly mixed in the VE/SB-S (53%), VE/HF (53%), and VE/FS (93%) groups. The failure modes observed in the LU groups were as follows: completely adhesive in the LU/C, LU/ C-S, LU/SB-S, and LU/HF-S groups, mostly adhesive in the LU/SB (87%) and LU/HF (80%) groups, completely mixed in the LU/FS group, and mostly mixed in the LU/FS-S (87%) group. The most frequently observed failure mode for VE was mixed failure (42%), followed by adhesive (37%) and cohesive (21%) failures. The most frequently observed failure mode for LU, on the other hand, was adhesive failure (71%), followed by mixed (27%) and cohesive (2%) failures (Figure 4).

Table 3: *Median (Min-Max) of the Shear Bond Strengths (MPa) of Ceramic-Resin Composites According to Different Surface Treatments*[a]

Surface Treatments	Vita Enamic		Lava Ultimate	
	Median	Min-Max	Median	Min-Max
C	0.00 Aa	0.00-0.65	0.00 Aa	0.00-0.00
C-S	0.20 Aa	0.00-1.79	0.00 Aa	0.00-0.51
SB	10.47 Ab	3.24-18.43	6.02 Bb	2.66-10.63
SB-S	18.64 Ac	11.60-25.36	6.32 Bb	2.62-12.04
HF	9.86 Ab	4.94-18.41	11.33 Ac	7.96-17.44
HF-S	22.99 Ad	14.02-32.64	12.20 Bc	9.12-20.24
FS	12.45 Ab	3.38-19.32	11.59 Ac	5.75-18.04
FS-S	25.55 Ad	9.06-35.30	18.14 Bd	4.26-31.49

Abbreviations: C, control; C-S, silane; SB, sandblasting; SB-S, sandblasting + silane; HF, hydrofluoric acid; HF-S, hydrofluoric acid + silane; FS, femtosecond laser; FS-S, femtosecond laser + silane.

[a] *Same uppercase letters in each row indicate no significant difference, while same lowercase letters in each column indicate no significant difference according to Mann-Whitney U-test (p>0.05).*

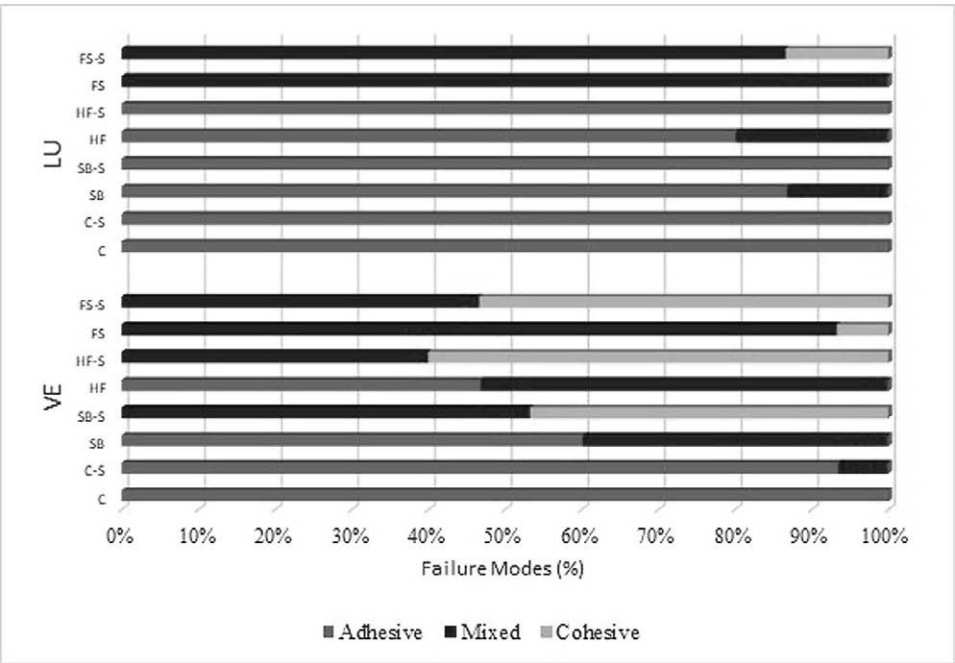

Figure 4. *Failure mode distributions according to surface treatment groups of Vita Enamic and Lava Ultimate.*

Correlation Analysis

There was a significant moderately negative correlation between surface roughness and SBS values in the VE/SB group (r=−0.63, p=0.012) and a moderately positive correlation in the VE/HF group (r=0.51, p=0.049); however, no significant correlation was observed in the other groups (p>0.05). On the other hand, prefailure was observed during the thermal cycle in the LU/C group; therefore, this group could not be evaluated in terms of correlation. The correlation results are displayed in Table 4.

DISCUSSION

The present study evaluated the effects of SB, HF, and FS treatments on the surface roughness of CRC materials and the effects of these treatments with and without silane coupling agent application on the bond strength. The first null hypothesis was rejected because FS treatment significantly enhanced surface roughness more than SB and HF treatments. The second null hypothesis was accepted for VE and partially accepted for LU because FS treatments did not enhance the SBS significantly except in the LU/SB group. Silanization increased the SBS significantly in the VE groups but did not increase it in the LU groups except the FS subgroup. Therefore, the third null hypothesis was rejected for VE and partially accepted for LU.

Table 4: *Correlation Coefficients (r) and p-Values of the Relationship Between the Average Surface Roughness (Ra/μm) of the Shear Bond Strengths (MPa) of Ceramic-Resin Composites According to the Spearman Correlation Test*

Surface Treatment/ Shear Bond Strength	Vita Enamic		Lava Ultimate	
	r	*p*	*r*	*p*
C	0.27	0.329	—	—
SB	−0.63	0.012	0.23	0.405
HF	0.51	0.049	−0.39	0.152
FS	0.50	0.058	0.41	0.125

Abbreviations: C, control; SB, sandblasting; HF, hydrofluoric acid; FS, femtosecond laser.

Cekic-Nagas and others[33] reported that resin cement and MDP-containing silane enhance the bond strength of CRC materials. In addition, MDP-containing resin cements reduce susceptibility to technical drawbacks and increase bond strength.[34] MDP-containing Panavia F 2.0 resin cement, which was used in the present study, was accepted as the gold standard by Behr and others,[35] and Kitayama and others[36] reported further improvement in the bond strength when it was combined with MDP-containing silane. Since the bond strength between ceramics and resin cement was evaluated in the present study, resin cement was applied directly to sample surfaces using an SDI SBS rig, as reported in previous studies.[26,37] Thus, the CRC cement interface was evaluated more reliably. Although the SBS test may cause cohesive failure with regard to nonuniform stress distribution and this may lead to faulty interpretations, it was preferred in the

present study because it can be easily and quickly applied with specific jigs standardizing the bonding area.[38] Similar to previous studies,[33,37,39] the SBS of the samples with prefailure during thermal cycling was considered to be 0 MPa in the present study.

Consistent with surface roughness values, the results of SEM images show that the surface texture of the VE/C group was rougher than that of the LU/C group. This result can be attributed to the fact that while VE contains microstructured silica particles, LU contains nanostructured silica and zirconia particles; moreover, it can be attributed to the differences in manufacturing processes and compositions of the materials. Although LU/SB showed higher roughness than VE/SB, LU/SB showed more irregular crater-like areas on the surface because of zirconia-silica particle networks. Consistent with its roughness values, VE/HF showed a more indented surface morphology than LU/HF. These results are consistent with those of previous studies.[40,41] Duzyol and others[42] reported that zirconia fillers and the resin matrix in the LU material were not affected by HF. On the other hand, the VE/FS and LU/FS groups showed similar surface textures, consistent with their roughness values. However, VE/FS exhibited a more porous surface morphology than LU/FS. The obtained images can be explained by the fact that LU has a resin matrix and VE a feldspathic ceramic matrix.

Because of its larger scanning area and versatility, a 3D profilometer can visualize surfaces where atomic force microscopy (AFM) proves to be ineffective.[43,44] The fact that the peak heights and pit depths observed in the FS groups are too deep and too high to be detected by AFM analysis justifies the use of 3D profilometry on surfaces with high roughness.[32,45] Akpinar and others[32] obtained peaks and pits with a range of 180 to 201 μm in zirconia ceramics using FS lasers with a 750-mW pulse power. The low heights and pits obtained in the present study can be attributed to the use of a low pulse power. Lorenzo and others[45] reported that pits of 15 to 90 μm provided higher bond strengths than pits of 120 to 180 μm. The pits of the laser groups in the present study were within the ideal range specified for bond strength.

Manufacturers recommend 5% HF and/or silane for VE[12] and SB (<50 μm Al_2O_3, <2 bar) and/or silane for LU.[5] Elsaka and others[40] reported that SB with 110 μm of Al_2O_3 or etching with 9% HF increases the bond strength and surface roughness of CRC and that silane further improves the bond strength of VE. On the other hand, Cekic-Nagas and others[33] reported that 10% HF did not increase the bond strength of CRC. In the present study, the SB treatment provided a higher surface roughness than the HF treatment for both materials; moreover, the surface roughness produced by the SB treatment was higher in the VE group than in the LU group, and the effectiveness of the SB, HF, and silane treatments was found to be consistent with the study by Elsaka.[40] The application of silane was found to be effective after HF for Vita VM7[46] and after HF and SB for IPS Empress 2.[21] Similar to the present study's finding, Elsaka[40] found the application of silane after SB and HF treatments to be effective only for VE and concluded that the differences in the results were due to different microstructural features and silica contents of the VE and LU materials. The effectiveness of silane application after SB and HF for VE can be attributed to the fact that the material has a silica-containing feldspathic matrix and that the selective removing effect of HF treatment removes the glassy phase in the ceramic and reveals more silica particles. In composite resins, the application of silane was not found to be effective after HF[47] and SB.[48] In the present study, in agreement with the above-mentioned results, the ineffectiveness of silane application after the SB or HF treatments for LU was attributed to the fact that the material has a composite matrix with resin content. The effectiveness of silane application after FS for LU can be attributed to the fact that more filler silica particles are released as a result of the removal of the resin structure by ablation. In this study, VE showed generally higher bond strength than LU. Thornton and Ruse[49] reported that VE exhibited superior mechanical properties and was less affected by storage in water than LU. Flury and others[50] reported that the SBS results for VE cemented with Panavia F 2.0 did not change even after storage for six months (37°C, 100% humidity); however, the SBS values for LU decreased. Cekic-Nagas and others[33] reported that thermal cycling reduced the bond strength of both materials and that the higher bond strength of VE was attributed to the fact that it absorbs less water because of its interpenetration phase. Thus, the hydrolytic stability of VE can be considered an important reason for the obtained results.

No studies or recommendations on CRC materials were identified in terms of FS or other laser surface irradiation. In this study, the FS treatment provided the highest roughness values, and the FS-S treatment yielded the highest SBS values for both materials. However, FS lasers have been reported

to produce homogeneous roughness without causing structural changes and thermal damage on the material surface by avoiding heat transfer on the surface using ultrashort laser pulses.[30] Akpinar and others[31] reported that FS laser provides homogeneous and regular surface roughness without generating cracks on the feldspar ceramic surface and that ablation patterns can also be controlled during laser irradiating using software. Although *in vitro* studies anticipate beneficial results about the prognosis of FS lasers since they compensate for negative properties of dental lasers, FS lasers have financial and dimensional disadvantages.[51,52] These disadvantages of FS lasers do not allow chairside applications in dentistry, whereas it is currently used in medicine and research laboratories. However, FS lasers in different configurations are expected to be introduced to dentistry and to increase in dental applications with FS lasers by reducing the cost of investment and simplifying their complex structure.[52-54] The effects on monomer chemistry and microcrack propagation of FS laser beams within the restorative materials were not encountered in the dental literature, and it seems that there is insufficient evidence for these issues.

Atsu and others[22] observed adhesive failures at lower bond strengths and mixed and cohesive failures at higher bond strengths. Oyague and others[55] also stated that mixed and cohesive failures are clinically preferable, as they maintain higher bond strengths compared to adhesive failures. In the present study, higher SBS (17 to 25 MPa) mostly exhibited mixed failure and rarely exhibited cohesive failure. Moreover, VE/HF, VE/FS, and LU/FS, which had lower SBS, showed mixed failures. These results may be due to the retention pattern of related surface treatments. In this study, cohesive failures were not observed in resin cement, as it was contained in the SDI SBS rig molds. Silane application increased the cohesive failure rate by increasing the bond strength in the VE material. In a similar study, Elsaka[40] most frequently observed mixed failure in both materials. On the other hand, Cekic-Nagas and others[33] most frequently observed adhesive failure in both materials. In the present study, adhesive failures (54%) were the most frequently observed failure mode.

In the present study, the surface roughness showed significant differences in the VE/SB, VE/HF, and VE/FS groups; however, their bond strengths were not significantly different. Although the LU/SB group showed higher roughness than the LU/HF group, its SBS value was significantly lower than that of the LU/HF group. This was attributed to the nonselective abrasive effect caused by SB treatment on LU because it may remove ceramic filler particles, which can be useful for bonding on composite surfaces. Although the LU/FS group showed higher roughness than the LU/HF group, their SBS values were similar. These results confirm the results of studies suggesting that the bond strength does not increase with surface roughness.[23,27,40] Oyague and others[55] reported that the type of resin cement has a higher impact on bond strength when compared to surface treatment methods. In the present study, surfaces with a lower roughness exhibited SBS values as high as those on surfaces with a higher roughness; this suggests that MDP-containing silane and cement increase SBS values of surfaces with a low roughness.

The limitations of the present study include the inability to fully simulate the mouth conditions and the inability to measure volume loss caused by surface treatments. Further studies are required to evaluate the effects of surface treatments on fracture resistance and discoloration, the effects of surface treatments with different sandblasting and femtosecond laser irradiation parameters and cements on bond strength, and the outcomes in the long-term clinical follow-up.

CONCLUSIONS

According to the results obtained in this study, the following conclusions were drawn:

1) FS surface treatment was the most effective method for both VE and LU in terms of surface roughness and bond strength to resin cement.
2) Although there was no significant difference between the surface roughness values of VE and LU, the bond strength to resin cement for VE was higher.
3) Silanization after surface treatments significantly increased the bond strength to resin cement for VE; however, it did not increase the bond strength to resin cement for LU except in the FS.
4) FS-S, HF-S, and SB-S surface treatments are recommended to condition the surface of VE restoration, and FS-S is recommended to condition the surface of LU restoration.

Acknowledgements

The authors are grateful to Mr Joshua Cheetham (chief technology officer of SDI Limited) for SDI SBS Rig and to Mr Mekki Kutlu (director of Armaera Digital) for the holder apparatus. This work was supported by the Office of Scientific

Research Projects Coordination of Kirikkale University (grant number 2014-20).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the University of Kirikkale.

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.

Note

This study has been presented in two parts as an oral presentation at the 47th Meeting of the Continental European Division of the International Association for Dental Research (CED-IADR) cohosted by the Scandinavian Division (NOF), October 16, 2015, Antalya, Turkey, and at the 20th International Congress of Esthetic Dentistry (EDAD), October 21, 2016, Istanbul, Turkey.

(Accepted 17 February 2018)

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In Vitro Effect of Innovative Desensitizing Agents on Dentin Tubule Occlusion and Erosive Wear

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Clinical Relevance

In-office desensitizing products are innovative and conservative methods for the decrease of dentin hypersensitivity due to its tubule occlusion properties. Depending on the diagnostic and pain related to the patient, the dentist is the professional responsible for indicating the appropriate treatment.

SUMMARY

Purpose: The purpose of this study was to evaluate the effects of four in-office desensitizing products on dentin tubule occlusion and erosive wear.

Methods: Dentin hypersensitivity was simulated by EDTA application for five minutes. The specimens were randomly allocated into five groups (n=11), according to treatment: No treatment - Control (C), Duraphat (DUR), Desensibilize Nano P (NP), ClinPro XT Varnish (XTV), and ClinPro White Varnish (CWV). They were then submitted to erosive/abrasive cycling for five days. After EDTA, treatment, and cycling, the specimens were analyzed with an environmental scanning electron microscope (ESEM) to verify the number of opened dentin tubules (ODT) which were counted by using ImageJ software, and with a profilometer to determine the surface curvature/loss. ESEM data were analyzed with two-way repeated measure analysis of variance and Tukey tests. For the profilometer, data were analyzed with Kruskal-Wallis, Tukey, and Mann-Whitney tests.

Results: After treatment, all groups showed lower ODT than the control, without signifi-

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DOI: http://doi.org/10.2341/17-284-L

cant differences between them. After cycling, the only group that showed lower ODT than the control was group XTV; however, it did not significantly differ from the other groups. For the profilometric analysis, there were significant differences in SL between the experimental times after treatment and after cycling for all groups ($p<0.05$). After cycling, no surface loss was detected in groups DUR and XTV, which presented a significantly different curvature than group NP and the control group, but not from group CWV. Surface loss was detected for the control and groups NP and CWV, without difference among them.

Conclusion: All desensitizing agents tested presented promising results concerning the obliteration of dentin tubules immediately after treatment. XTV was the only desensitizer capable of preventing the reopening of the tubules after the erosive/abrasive challenges. XTV and DUR presented a protective effect against dentin erosive wear.

INTRODUCTION

Dentin hypersensitivity (DH) is a common condition among patients around the world, and its characteristics are well known and widely reported in the literature.[1-3] There are many etiologic factors related to DH and denudation of the root surface with loss of the overlying cementum and periodontal tissues and the removal of the enamel because of wear being commonly associated with DH.[4] In this latter condition, it is likely that the impact of erosive acids and toothbrushing, depending on the degree of the abrasivity of the toothpaste, can open the dentin tubules, thus leading to DH.[5]

As the theory proposed by Brännström is the most widely accepted to explain the mechanism of pain in DH,[6-8] one of the main strategies to treat this condition consists in sealing the dentinal tubules, thus preventing fluid flow. There are currently a large number of commercially available desensitizing agents.[9-13] Among the in-office options that can be cited are adhesives, glass ionomer cements and sealants, as well as topical application of products containing sodium fluoride, tin fluoride, potassium nitrate, oxalates, calcium phosphate, oxalic acid (phytocomplexes), arginine/calcium carbonate, and bioglass (calcium phosphosilicate and sodium).[13]

Varnishes containing sodium fluoride are one of the most widely used products for the treatment of DH. Fluoride possibly acts by precipitating insoluble calcium fluoride within the dentinal tubules.[14] Clinical studies show that varnishes have beneficial effects in the treatment of DH[15,16]; however, according to a recent systematic review, its clinical effectiveness was considered only as limited.[2] Clinpro White Varnish (3M ESPE, St. Paul, MN, USA) is an example of a fluoride varnish, which, in addition to sodium fluoride (5%), contains tricalcium phosphate (TCP).[17] According to the manufacturer, the material releases calcium and fluoride ions when in contact with saliva, optimizing the formation of calcium fluoride up to a period of 24 hours. There is not much data evaluating the effectiveness of this varnish on tubule occlusion, especially under erosive and abrasive conditions. Tosun and others observed that at the end of a pH cycling the material remained at the dentin surface, partially occluding the dentinal tubules.[18]

Also containing calcium and phosphate in its composition, nano hydroxyapatite pastes (such as Desensibilize Nano P, FGM Dental Products, Joinville, SC, Brazil) were recently suggested as another treatment option for DH.[19-21] It was hypothesized that these pastes would promote tubule occlusion by the deposition and/or penetration of nano-sized particles onto or into the dentin tubules.[21] Clinical trials have shown that these pastes are effective against DH.[21-23] It has been observed that the particulate nano calcium phosphate exhibits mechanical obliteration properties twice as good as the traditional calcium phosphate compositions[24]; however, a recent *in vitro* investigation observed that the deposits formed by a hydroxyapatite paste is not resistant to acidic and mechanical challenges when compared to two varnishes.[25]

Another innovative product used for DH is a resin-modified glass ionomer, Clinpro XT Varnish (3M ESPE, St. Paul), which the manufacturer claims provides a specific coating of fluoride release for more than six months. The varnish also releases calcium and phosphate in a controlled manner.[26] In a clinical trial, it was observed that this material could reduce DH through a period of four weeks, being more effective than the resin-based material Gluma Desensitizer (Kulzer, Hanau, Germany).[25] An *in vitro* study found that it could sustain tubule occlusion even after seven days of erosive and abrasive challenges.[25] The manufacturer also claims that the product can be used to prevent dental erosion, but there is not much information about this in the literature.

Considering that these innovative desensitizing agents contain fluoride, calcium, and phosphate in their composition and that they are applied to the dentinal surface promoting a mechanical barrier, it is reasonable to suppose that they can also offer some protection against dentin erosive wear. This would be of utmost importance, because dental erosion and abrasion are strongly associated with the etiology and development of DH.[27] Thus, the objectives of this study were as follows: 1) to investigate, *in vitro*, the efficacy of four desensitizing agents in promoting tubule occlusion, as well as its resistance to erosive/abrasive challenges and 2) to evaluate their effect against dentin erosive wear.

The null hypotheses tested were the following: 1) there would be no difference among the products regarding the occlusion of dentinal tubules post treatment; 2) the products would not differ in their ability to promote tubule occlusion after an erosive/abrasive challenge; and 3) there would be no difference in dentin erosive loss among the groups after cycling.

METHODS AND MATERIALS

This study followed a completely randomized design with two experimental factors: desensitizing treatment was five levels: no treatment-negative control (C), Duraphat (DUR), Desensibilize Nano P (NP), ClinPro XT Varnish (XTV), and ClinPro White Varnish (CWV). Experimental time was three levels for tubule counting analysis (after EDTA, after treatment, and after cycling) and two levels for the profilometric evaluation (after treatment and after cycling). The factors were tested in an erosion-abrasion-remineralization model using human specimens (n=11 per subgroup). The response variables were dentin surface loss (SL, in micrometers, determined with optical profilometry) and the number of opened dentinal tubules (ODT; tubule counting was performed with ImageJ software [National Institutes of Health, Bethesda, MD, USA] on scanning electron microscopy images).

Sample Preparation

Eighty sound human third molars were collected. Human third molars were used in this study after the approval of the Local Ethics Committee (CAAE64008417.0.0000.0075). Teeth were cleaned with Gracey curettes 11-12 and 13-14 and Robinson's brush at low speed using a mixture of pumice and water, ending with an air/water spray. The roots were separated from the crowns using a water-cooled diamond disc (KG Sorensen, Barueri, São Paulo, Brazil) and stored in 0.1% thymol solution at 4°C until the beginning of the experiment. Dentin specimens 4 × 4 × 2 mm were cut from the roots and polished with a series of abrasive discs cooled with water (grit# 800, 1200, 2400, and 4000; Buhler, Uzwil, Switzerland). Between each polishing step, the samples were cleaned with distilled water in an ultrasonic cleaner (Digital Ultrasonic Cleaner CD-4820, Kondortech, São Carlos, Brazil) for five minutes to remove any debris. To simulate a hypersensitive dentin, the specimens were immersed in 17.5% EDTA solution for five minutes to remove the smear layer and open the dentin tubules. After that, the specimens were sonicated in distilled water (Digital Ultrasonic Cleaner CD-4820, Kondortech, Sao Carlos, Brazil) for five minutes to remove any debris.[28]

Treatments

After opening the dentinal tubules, the samples were randomly allocated into five experimental groups (n=11), according to their respective treatments (Table 1). All specimens received adhesive unplasticized polyvinyl chloride tapes (UPVC, Graphic Tape, Chartpak, Leeds, MA, USA) on their polished surfaces, leaving a central window of 4 × 1 mm exposed to receive the treatments and two lateral areas of 4 × 1.5 mm as control surfaces. The treatments were applied following the manufacturer's instructions, as described in Table 1. The negative control group received no treatment.

Abrasive/Erosive Challenges

For all groups, a modified five-day erosion-abrasion-remineralization model proposed by Scaramucci and others was used.[29] Erosive challenges were performed with a 0.3% citric acid solution (pH ≅ 2.6). The specimens were immersed in the citric acid solution for two minutes, four times a day, without stirring and at room temperature. Between the challenges, there was a 60-minute immersion in artificial saliva (1.649 mmol/L $CaCl_2 \cdot H_2O$, 5.715 mmol/L $KH_2 PO_4$, 8.627 mmol/L KCl, 2.950 mmol/L NaCl g/l.92 mmol/L Tris buffer, pH adjusted to 7 with HCl). After each episode of erosion, the specimens were rinsed with distilled water and gently dried with absorbent paper.

Tooth brushing was performed twice a day for 15 seconds in the middle of the first and last remineralization periods using electric brushes (Oral B Professional Care 3000f, Procter & Gamble, Cincin-

Table 1: *Description of the Densensitizing Products, Composition of the Agents Used in This Study, and Manufacturer's Application Instructions*

Product	Manufacturer	Composition	Protocol
Negative control			No surface treatment after sample preparation with EDTA
Clinpro white varnish	3M ESPE	Sodium fluoride (5%), tricalcium phosphate (TCP), xylitol	Apply in a thin layer over treatment area(s) with sweeping, horizontal brush strokes
Clinpro XT varnish	3M ESPE	Part A: glass particles of silanized fluoroaluminosilicate, HEMA, water, BIS-GMA, and silanized silica Part B: copolymer of polyalkenoic acid, water, HEMA and calcium glycerophosphate	Mix the components for 15 seconds; application of the varnish in a thin layer on tooth surface light-curing for 20 seconds and surface cleaning with a moistened pellet
Desensibilize nano P	FGM	Nanometric calcium phosphate, sodium fluoride (approximately 2%) and potassium nitrate 5%.	Active application for 10 seconds with felt disc at low speed; five minutes waiting time and excess removal
Duraphat	Colgate–Palmolive	Sodium fluoride (5% w/v) in an alcoholic solution of natural resins	With the surface dry, apply the material in a thin layer (brush, applicator or probe)

nati, OH, USA) equipped with a pressure alert that signals when the pressure reaches the value of 2.5 N. The brush head was positioned on a stabilizing device parallel to the surface of the specimens until the pressure alert was switched on. For the tooth for all groups, brushing was performed with a slurry made from Colgate Maximum Anti-caries Protection dentifrice and artificial saliva (1:3 w/w). The total exposure time of the specimens to the dentifrice slurries in each brushing episode was two minutes. A single operator performed the toothbrushing procedures. During the night, specimens were stored in a humid environment at 4°C.

Profilometric Analysis

After EDTA treatment, all specimens were analyzed with optical profilometry (Proscan 2100, Scantron Ltd, Venture Way, Taunton, UK) to discard specimens with curvature values higher than 0.3 μm, for sample standardization. The other readings were performed after treatment and at the end of cycling. The specimens were left to dry for 10 minutes before each profilometric analysis.[30] The instrument sensor scanned an area 2 mm long (x-axis) by 1 mm wide (y-axis), located at the center of the specimen. The equipment was set to go through 200 steps on the x-axis, with each step measuring 0.01 mm. On the y-axis, there were 20 steps measuring 0.05 mm each. Using a specific software (Proscan Application software version 2.0.17, Scantron Ltd), the lesion curvature or depth was calculated based on subtracting the average height of the test areas from the average height of the reference surfaces. The result was expressed in micrometers.

Environmental Scanning Electron Microscopy (ESEM) Evaluation

After EDTA treatment, all specimens were analyzed by ESEM (Hitachi TM3000, Hitachi, Tokyo, Japan) to verify, qualitatively and quantitatively, the number of opened dentin tubules. Representative micrographs were taken at ×2000 in the center of each specimen. No sample preparation was required. All specimens were qualitatively and quantitatively re-evaluated after treatments and cycling. In the qualitative assessment, micrographs had their surface characteristics evaluated and checked for patency and occlusion of the dentin tubules. The quantitative assessments were performed using the image analysis software program, ImageJ (National Institutes of Health), to standardize the counting of opened dentin tubules.[31,32]

Statistical Analysis

Data were analyzed for normal distribution and homoscedasticity with Shapiro-Wilks and Brown-Forsythe tests, respectively. For the ODT data, these assumptions were satisfied; therefore, comparisons among groups were performed with two-way repeated-measures analysis of variance (ANOVA) and Tukey tests. For surface loss, data did not follow a normal distribution; thus, comparisons among groups within experimental times were performed with Kruskal-Wallis and Tukey tests. Comparisons between experimental times (after treatment and

Table 2: *SEM Evaluation: Mean Values of Opened Dentinal Tubules*

Groups	After EDTA, Means (SD)	After Treatment, Means (SD)	After Cycling, Means (SD)
Control	129.09 (47.97) Aa	104.64 (29.42) Aab	76.00 (24.43) Ab
DUR	134.73 (53.12) Aa	0 (0) Bc	68.73 (50.73) ABb
XTV	129.64 (48.02) Aa	0 (0) Bc	31.91 (44.43) Bc
CWV	132.18 (46.48) Aa	0 (0) Bc	74.09 (42.12) ABb
NP	136.54 (49.72) Aa	4.73 (9.06) Bc	74.73 (31.85) ABb

In columns, uppercase letters indicate differences among groups at each experimental time. In rows, lowercase letters indicate differences amongst experimental times for the same treatment group.

after cycling) within groups were performed with the Mann-Whitney test. The software Sigma plot 12 (Systat Software Inc, San Jose, CA, USA) was used for all calculations. The significance level was set at 5%.

RESULTS

For ODT, there were significant differences among the levels of the factors treatment ($p<0.001$) and experimental times ($p<0.001$) and in the interaction between them ($p<0.001$).

Relative to the factor treatment after EDTA, there were no significant differences in the number of ODT among the groups. After treatment, all the groups showed lower ODT than the control, without significant differences between them. After cycling, the only group that showed lower ODT than the control group was group XTV; however, it did not significantly differ from the other groups. All the groups, except XTV, did not significantly differ from the control.

Regarding the factor experimental times, for the groups CWV, DUR, and NP, ODT was lower after treatment, followed by after cycling and then after EDTA. For the control, the lowest ODT was observed after cycling, but it was no different from ODT after treatment, which in turn did not differ from after EDTA. For group XTV, the highest ODT was observed after EDTA, and the ODT from after treatment and after cycling was not significantly different.

The means (SD) of open dentin tubules according to each DH treatment in all experimental times are shown in Table 2. Representative images of the groups in each time, are shown in Figures 1 and 2. Also, an example of ImageJ software (National Institutes of Health) analysis of opened dentinal tubules may be seen in Figure 2.

For the profilometric analysis, there were significant differences between the experimental times after treatment and after cycling for all groups ($p<0.05$).

After treatment, groups DUR, XTV, and CWV presented the highest curvature, with no significant difference among them, indicating the presence of a layer of the material on their surface. The curvature of the control group did not differ from that of group NP, which in turn was no different from group CWV. After cycling, no surface loss was detected in groups DUR and XTV, which presented significantly different curvature than group NP and the control group, but not from group CWV. Surface loss was detected for the control group and groups NP and CWV, with no differences among them.

Medians (interquartile interval) of dentin surface loss for the groups in each experimental time are shown in Table 3.

DISCUSSION

In the present study, all desensitizer agents were capable of obliterating the dentinal tubules immediately after their application, making them suitable approaches for the treatment of DH. In view of this result, the first null hypothesis was accepted. However, only group XTV showed resistance to the erosive/abrasive challenges, sustaining tubule occlusion after cycling; therefore, the second null hypothesis was rejected.

Table 3: *Profilometer Evaluation: Median Values of Dentin Surface Loss*

Groups	After Treatment, Median (25%/75%)	After Cycling, Median (25%/75%)
Control	0.13 (0.07/0.25) C	−7.653 (−9.18/−5.72) B
DUR	151.89 (108.56/311.89) A	3.769 (−2.00/56.97) A
XTV	118.67 (75.19/153.12) A	0.143 (−4.44/110.72) A
CWV	66.21 (48.35/132.51) AB	−4.110 (−5.51/−2.79) AB
NP	2.84 (1.20/6.68) BC	−5.374 (−6.34/−4.46) B

All test groups showed statistically significant differences between experimental times after treatment and after cycling ($p<0.05$).

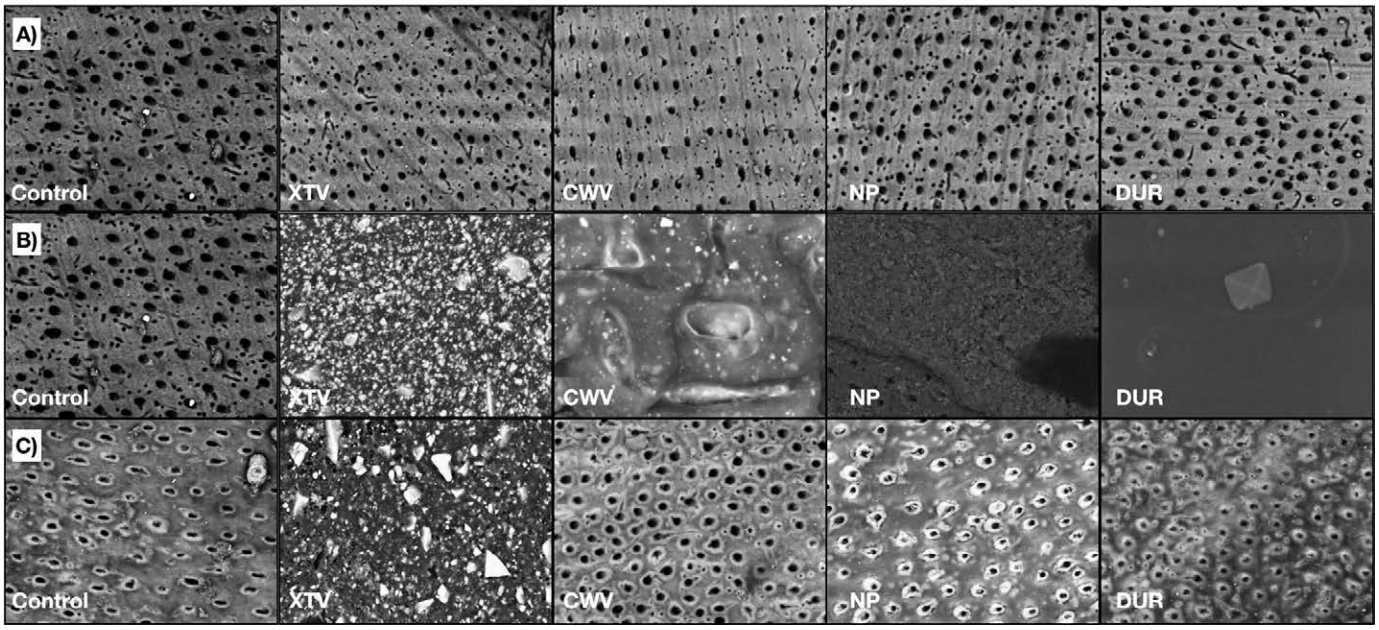

Figure 1. *(A) Dentin surface after EDTA. Control group, XTV group, CWV group, NP group, and DUR group. (B) Dentin surface after treatment. Control group, XTV group, CWV group, NP group, and DUR group. (C) Dentin surface after erosion/abrasion cycling showing a visibly worn treatment layer with dentin exposure. Control group, XTV group, CWV group, NP group, and DUR group.*

ClinPro XTV varnish (XTV) is a resin-modified glass ionomer and, as such, can be used as a sealant for class V lesions. Its efficiency may be explained by the action of its components, such as the polyalkenoic acid copolymer, which provides chemical adhesion to dentin by ionic bonding with the hydroxyapatite calcium, the prevalent mineral of dentin.[33] The lack of surface loss observed for XTV after cycling could be due to the presence of a coating of material over the dentinal surface, which prevented direct acid contact, and the action of toothbrushing. Compared with the profilometric analysis performed after treatment, the surface curvature of this group was reduced after cycling, suggesting that, under the conditions of this study, the material underwent some wear, but not up to a point of allowing surface loss. Nevertheless, it should be mentioned that, in a few specimens, a complete detachment of the

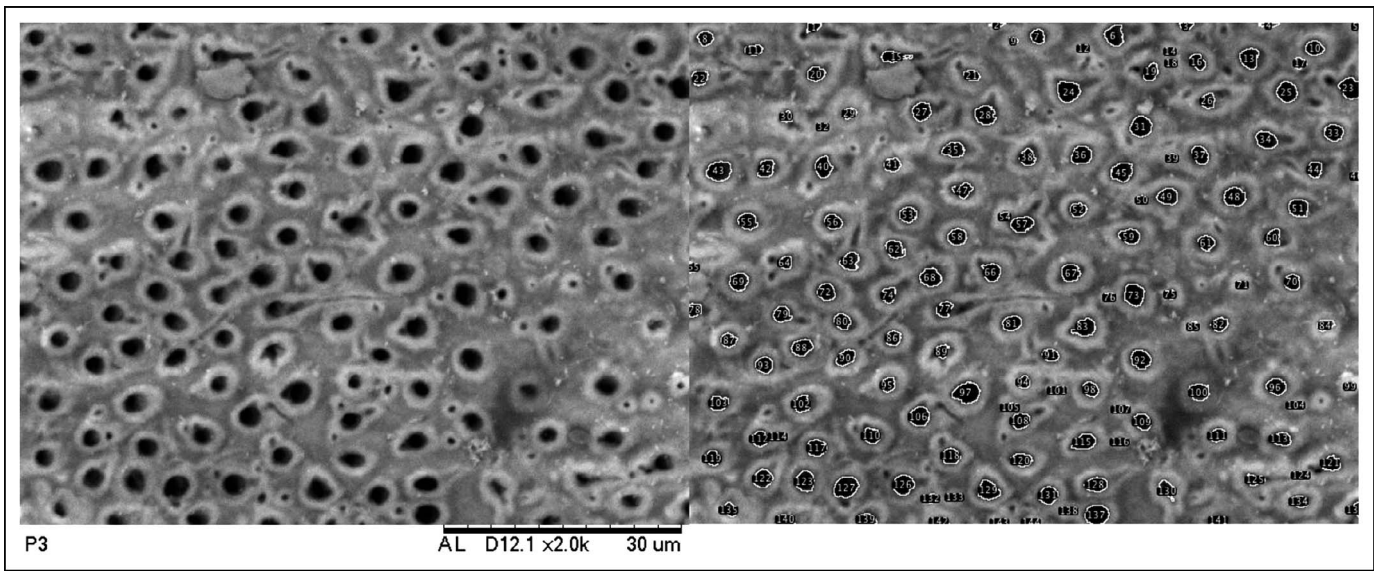

Figure 2. *Example of ImageJ software count of opened tubules.*

material off the surface was observed after cycling, thus producing the large variation found in the data from this group. In view of these results, further investigations are needed to evaluate the impact of acidic and mechanical challenges on the adhesion of the material to the dentin, especially in the long term. A previous investigation observed that the shear bond strength of XTV to dentin was lower than some resin-based coatings.[34]

In addition to promoting a physical barrier at the dentin surface, XTV is able to release fluoride, calcium, and phosphate to the aqueous medium; the two latter are a result of the presence of calcium glycerophosphate in its composition.[35,36] Such mechanisms could be observed in previous studies.[26,37] In the investigation of Virupaxi and others,[37] fluoride released in artificial saliva by XTV was evaluated for a period of six months. The authors observed that the material released 18.78 ppm of F within the first week, a value that dropped to almost half after one month, but that was kept relatively stable for six months. It must be noted, however, that in this study, the material was applied to the enamel and not the dentin. In Zhou and others,[26] the authors observed that XTV was more effective than a fluoridated varnish in promoting enamel remineralization, an observation that was attributed to the presence of calcium and phosphate in its formulation. In view of these findings, in the case of the present study, the authors also suggest that XTV released calcium, phosphate, and fluoride to the acidic solution during the erosive challenge, increasing its saturation regarding tooth minerals, thereby reducing the demineralization rate. Similarly, it could have released these ions when in the saliva, enhancing remineralization.

Another material that was able to significantly reduce dentin loss after cycling, although not being able to promote a significant tubule occlusion, was the fluoridated varnish Duraphat (DUR). A NaF varnish was included in the present study as a positive control, as it is commonly applied in dental practices for the treatment of DH.[2] Its fluoride-releasing mechanism is widely described in the literature as the creation of a barrier by precipitating calcium fluoride (CaF_2) on the tooth surface, which blocks the patent dentinal tubules and hence reduces permeability and hypersensitivity.[15,16,38,39] Similar to XTV, the ability of DUR to reduce dentin loss can be attributed to its persistence at the dentin surface until the end of the cycling, although some detachment was also observed. In addition, the deposition of a CaF_2-like material may also have contributed to the dentin's protection.[40] However, the occluding ability of DUR was not significantly higher after cycling than the control. It could be suggested that not only complete, but also partial, detachment of this varnish occurred throughout the cycling, which resulted in the opening of some dentinal tubules. To corroborate this finding, the number of ODT for this group was lower after cycling than after EDTA, indicating that at least a portion of the dentinal tubules remained occluded by the varnish. This theory is in agreement with the observations of West and others, who stated that the effectiveness of varnishes in DH appears to be limited to the duration in which they remained on the tooth surface,[2] which was estimated to be 24 hours *in situ*. However, it must be mentioned that, in DUR, although the software considered some tubules opened, they seemed to have their diameter reduced compared with the control (Figure 3). This must be the result of the deposition of the CaF_2-like material, which may exert a clinical impact on DH.

Due to the presence of calcium and fluoride in its formula, it was expected that CWV would behave better than DUR regarding tubule occlusion, possibly by the formation of more CaF_2-like deposits, but this was not observed. Although CWV was able to promote some tubule occlusion that did not return to the values after EDTA after cycling, at that time its number of ODT did not differ from the control.[17,41,42] Similar to DUR, it can be hypothesized that the cycling promoted partial removal of CWV from the surface, exposing dentin tubules. Nonetheless, opposite of DUR, the tubules with CWV do not appear with a reduced diameter. Regarding surface loss, it can be suggested that CWV has lower adhesion to the dentin compared with DUR due its lack of protection against erosive wear. In this context, it must also be taken into account that the layer of material induced by CWV was thinner than DUR, as can be seen in the surface profile obtained after treatment. This can also influence the comparison. Concerning the formation of CaF_2-like deposits, in previous investigations, the amounts of fluoride, calcium, and phosphorus released by different varnishes in lactic acid were evaluated. It was observed that within 24 hours of exposure, the calcium release of CWV was no different from DUR, but after 48 hours, it was significantly higher than DUR. Phosphorus release was very low for CWV, but its fluoride release was almost four times higher than DUR in both experimental times.[43] The authors suggested that the low phosphorus found for CWV could be related

to the low amount of beta tricalcium phosphate (TCP) added to the varnish or to the low solubility of tricalcium phosphate. If that is indeed the case, it could also help to explain why CWV did not behave better than DUR under the conditions of this investigation.

In this study, Desensibilize Nano P (NP) was effective in occluding the dentinal tubules immediately after application; however, it did not show a resistance to the cycling procedures, despite the manufacturer's claim that NP imparts greater stability and resistance to the acid challenge because of the crystalline form of its mineral content. NP resulted in a heterogeneous pattern of obliteration, also observed by Canali and others.[36] This might have occurred because of its lower viscosity, thus resulting in a thinner layer of product over the tooth surface. Another explanation would be related to the lower concentration of fluoride in NP compared with the other products tested. It has less than half of the fluoride content than Duraphat and ClinPro White Varnish (9000 versus 22,600 ppm F), which may have impacted its ability to form CaF_2-like material, as higher concentrations induce more deposition of these globular structures.[44] It is possible that a greater number of applications would result in a more effective occlusion, as shown in a previous clinical study.[45] This result is in accordance with a previous *in vitro* report, which showed that NP was less effective in reducing dentin permeability than XTV and a fluoridated varnish immediately after application and after seven days of erosive-abrasive cycling.[43] Nevertheless, that study is in disagreement with a previous investigation that tested a different nano hydroxyapatite paste and found that the precipitates formed by the paste were resistant to an erosive challenge of one minute with 6% citric acid.[46] The more aggressive erosive challenge performed in the present study (two-minute immersion in 0.3% citric acid, four times a day for five days) might explain these different results. Clinically, NP demonstrated the ability to reduce DH for up to three months.[21] In this case, the role of fluoride and potassium nitrate as active agents included in the NP should also be considered. It has been discussed that potassium salts are able to inactivate intradental nerves, contributing to the reduction of DH. However, this principle has never been confirmed.[21] Concerning surface loss, NP did not show any protective effect. Not much data exists about the effect of nano hydroxyapatite pastes on dental erosion.

This study used an erosion-abrasion cycling model with the main objective of simulating the clinical situation of individuals reporting DH, because dietary acids and toothbrushing are known to be capable of opening and enlarging dentin tubules, initiating the condition, or reducing the effectiveness of the treatments. The cycling protocol was adapted from Scaramucci and others and attempted to simulate the situation of patients with a high frequency of acidic beverage consumption.[29,47] The literature has reported that in the mouth, the maximum time that the pH remains low is about two minutes, and extrapolation of this condition may modify the eroded surface to an unrealistic state.[47,48] Toothbrushing abrasion occurred twice daily, in an endeavor to simulate a realistic daily oral hygiene habit.[27] Toothbrushing was performed with an electric toothbrush, fixed in a specific device that standardized the brush movement over the specimen and controlled the brushing force at 2.5 N, which is within the range of force recommended for erosion-abrasion studies.[31,49] Colgate Maximum Anti-caries Protection was chosen for toothbrushing because it is a regular 15,000 ppm F (as NaF) toothpaste, without any desensitizing claim. The slurry used was prepared with artificial saliva, which has calcium in its composition. This could enhance the action of the desensitizing agents tested, as well as explain the partial occlusion of some tubules in the control group after cycling compared with after EDTA. Because artificial saliva was used, the enzymatic and microbiological effects of human saliva could not be expected.

To properly simulate clinical conditions, the desensitizer agents were applied according to the manufacturers' instructions. A limitation of this methodology was that, as each product presented a different consistency, the layers applied on each specimen were not uniform and standardized. In this sense, care should be taken when extrapolating the findings of this study to the clinical scenario, but it can provide information and a basis for further analysis under more realistic clinical conditions.

CONCLUSION

Considering the limitations of this *in vitro* investigation, it can be concluded that all desensitizing agents tested presented promising results concerning the obliteration of dentin tubules immediately after treatment. ClinPro XT Varnish was the only desensitizer capable of inhibiting the reopening of the tubules after erosive/abrasive challenges. Clin-

Pro XT Varnish and Duraphat presented a protective effect against dentin erosive wear.

Acknowledgements

The authors thank CAPES (Coordination of Superior 2 Level Staff Improvement, Social Demand grants) and FAPESP (State of São Paulo 3 Research Foundation, Grants #2016/20935-2).

Regulatory Statement

This study was conducted in accordance with all the provisions of the approval of the Local Ethics Committee guidelines and policies of the Faculty of Dentistry of the University of Sao Paulo. The approval code for this study is CAAE64008417.0.0000.0075.

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.

(Accepted 31 January 2018)

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Effect of Mouth Rinse Treatments on Bleached Enamel Properties, Surface Morphology, and Tooth Color

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Clinical Relevance

Mouth rinses are popular agents used in combination with esthetic treatments. Some mouth rinses containing different active ingredients can affect bleached enamel properties.

SUMMARY

Objective: To evaluate, *in vitro*, the effect of mouth rinse exposure on bleached enamel.

Methods: Enamel/dentin bovine blocks (4×4×2 mm) were bleached with 35% hydrogen peroxide (HP) and were submitted to immersion twice daily for 14 days with different rinses (n=10), including those involving: distilled water (C [control]), 225-ppm NaF (FM, Colgate Plax Classic), essential oil (EM, Listerine Tartar Control), 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening), and 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate® Luminous White). The specimens were stored in a remineralizing solution during all experiments. Analyses of color (ΔE, L*, a*, b*) and roughness (Ra) were performed at the baseline, after HP, and after exposure to the rinse. The cross-sectional microhardness (CSMH) and images by scanning electron microscopy (SEM) were assessed at the end. The data were subjected to analysis of variance (ANOVA) (ΔE), repeated measures ANOVA (Ra), and split-plot ANOVA (CSMH), followed by the Tukey test. The L*, a*, and b* values were analyzed by generalized linear models (α=0.05).

Results: Color changes were not statistically different in the groups. Ra increased in all groups after bleaching; however, it was reestablished in C, FM, and HPM+F and increased in EM after 14 days of the rinse. EM and HPM reduced the CSMH values differing from C and promoted alterations on the enamel surface visualized by SEM.

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DOI: http://doi.org/10.2341/17-250-L

Conclusion: The mouth rinses did not affect the whitening efficacy or promote benefits on bleached enamel properties. Moreover, the 1.5% hydrogen peroxide– or essential oil–based mouth rinses affected the bleached enamel properties, promoting an alteration in morphologic surface and mineral loss in depth.

INTRODUCTION

Since the demand for esthetic dentistry has extensively increased recently, treatment protocols for discolored teeth have been developed, with tooth bleaching becoming a common treatment that promotes improvement in the appearance of the smile.[1] Categorically, there are different methods and approaches to whitening treatment:[2] 1) dentist-supervised or at-home bleaching using low-concentration bleaching agents in a high-frequency regimen, 2) in-office or power bleaching, generally applying relatively high-concentration agents on the dental substrate, and 3) over-the-counter (OTC) whitening agents represented by mass market bleaching products containing low concentrations of whitening agents that are self-applied to the teeth via mouthwash, toothpaste, or strips. In general, the action mechanism most likely involved in tooth bleaching is related to the properties of hydrogen peroxide (HP) as an oxidizing agent that can break down the chromogen and remove the pigments from the structure of enamel or dentin through diffusion, indirectly promoting the reduction of light absorption.[3] The reduction in light absorption produces a significant reduction in the yellowness of dentin and an increase in the whiteness of the tooth.[4]

Tooth bleaching has been widely indicated since this procedure is considered esthetic, relatively safe, and effective.[1,2] Scientific evidence[5-9] shows that changes in the morphology and properties of dental tissues can happen, especially related to an unspecified oxidative effect of HP that could act in the inorganic and organic composition of the tooth.[9] In order to eliminate the side effects of dental bleaching, different products and vehicles, such as fluoride in gel or toothpaste, have been suggested for use before or after treatment.[10-12] There are no investigations regarding the effects of compounds incorporated in different commercial mouth rinses on bleached enamel with 35% HP.

Mouth rinses are very popular oral hygiene agents, and combinations of different preventive and therapeutic agents are commercially present. Considering the OTC products, whitening mouth rinses appeared on the market as an alternative to treatment for tooth discoloration, with a lower cost than traditional guided approaches. The whitening effectiveness of these agents is rarely discussed and is controversial in the literature, especially due to the lack of clinical studies that validate the effectiveness and safety of these products. Previous *in vitro* studies described no efficacy,[13] slight bleaching effectiveness before 45 days of use,[14] or similar color alteration compared with 14 days of at-home bleaching therapy when used for 12 weeks.[15] Moreover, a recent study[16] demonstrated that the whitening efficacy of some OTC mouth rinses may increase the longevity of at-home whitening outcomes over time. There are few studies regarding the use of OTC whitening associated with dental bleaching or evaluating the effects of use of commercial mouth rinses containing different active principles on bleached enamel, mainly in relation to enamel properties, effectiveness of bleaching, and color stability of treatment.

In addition to OTC products, other agents are frequently incorporated into mouth rinses in order to decrease or prevent biofilm-associated oral diseases as an adjunct to mechanical oral hygiene measures. Essential oil mouth rinses are very popular agents used in a combination with thymol, menthol, eucalyptol, and hydroalcoholic vehicles. In particular, blue-colored alcohol and essential oil–containing mouth rinses have been shown to be capable of causing color change of enamel.[17] No investigations have evaluated the effects of these compounds on bleached enamel, which is necessary since it has been suggested that the low pH of rinses can promote some enamel erosion.[18,19]

Further investigation is warranted considering the absence of studies investigating the effects of different commercial mouth rinses on bleached enamel, which may have its mineral content altered.[8] The aim of this study was to investigate the effects on enamel of mouth rinses with different active agents used after in-office dental bleaching, using analysis of color, cross-sectional microhardness, and surface roughness in order to evaluate the properties and morphology of bleached enamel. The null hypotheses tested were that 1) in-office dental bleaching would not affect the enamel properties of the surface and subsurface, 2) the use of a mouth rinse after in-office dental bleaching would not affect the whitening efficacy or promote the incorporation of pigments, and 3) the use of a mouth rinse after dental bleaching would not affect the bleached enamel properties of the surface and subsurface.

Table 1: *Components of Products Used According to the Manufacturer's Information*

Product	Manufacturer	Composition	Color	pH[a]
Whiteness HP (bleaching agent)	FGM (Santa Catarina, Brazil)	35% hydrogen peroxide, carbopol, glycol, and water	Initial = red; after 15 min = colorless	Initial = 5.64; after 15 min = 4.87
Colgate Plax Classic (FM)	Colgate-Palmolive (São Paulo, Brazil)	225-ppm NaF (sodium fluoride), triclosan 0.03%, PVM/MA copolymer, 0.20% gantrez, alcohol, water, sorbitol, glycerin, sodium lauryl sulfate, disodium phosphate, sodium hydroxide, sodium saccharin, CL 16035	Red	6.11
Listerine Tartar Control (EM)	Johnson & Johnson (São Paulo, Brazil)	21.6% alcohol, 0.064% thymol, 0.092% eucalyptol, 0.06% methyl salicylate, water, n-propranolol, sorbitol, polaxamer 407, peppermint flavoring, benzoic acid, sodium benzoate, sodium saccharin, zinc chloride, blue dye FD&C	Blue	4.30
Colgate Plax Whitening (HPM)	Colgate-Palmolive	1.5% hydrogen peroxide, water, sorbitol, ethyl alcohol, poloxamer 338, polissorlato 20, methyl salicylate, menthol, saccharin sodium, CI 42090	Light blue	4.07
Colgate Luminous White (HPM+P)	Colgate-Palmolive	2% hydrogen peroxide, tetrapotassium pyrophosphate, tetrasodium pyrophosphate, 225-ppm NaF, zinc citrate, glycerine, propylene glycol, phosphoric acid, saccharin sodium, sucralose, flavor	Light blue	6.92

[a] *The pH was determined in triplicate using a pH meter (Procyon, São Paulo, Brazil).*

METHODS AND MATERIALS

Sample Preparation

Enamel/dentin blocks ($4 \times 4 \times 2$ mm), with 1 mm of enamel and 1 mm of dentin, were obtained from the middle third of the buccal surface of sound bovine incisor teeth that were stored in a 0.01% thymol solution at 4°C for 30 days until use. The sections were obtained using a low-speed water-cooled diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA). The samples were subsequently serially ground with 600-, 1000-, and 2000-grit SiC papers (Buehler) and polished with cloths and diamond spray (1, 0.5, and 0.25 μm, – Buehler). The samples were placed in an ultrasonic machine for 10 minutes (Marconi, Piracicaba, Brazil) to remove residues in order to obtain a standardized enamel surface. All surfaces of the blocks, except the enamel surface, were protected with acid-resistant varnish. During the experiment, all prepared samples were stored in a remineralizing solution containing 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 μg F/mL, and 0.1 M Tris buffer at pH 7.0[20] that was renewed every day of the study. The initial L* values of each sample were used to stratify and allocate specimens into all groups, aiming to reduce the initial variability among the treatments. The evaluation method of the L* coordinate is described in the "Color Measurements" section.

Bleaching Procedure

The bleaching treatment was performed using an agent of high HP concentration (35% HP, Whiteness HP, FGM, Joinville, Brazil), according to the manufacturer's instructions. Information on the product and its pH are shown in Table 1. All prepared samples were stored in a remineralizing solution for 24 hours prior to the bleaching procedure. The bleaching agent was applied three times for 15 minutes on the enamel surface. After the procedure, the samples were washed with distilled water, and a daily exposure regime with mouth rinses was initiated.

Mouth Rinse Application Protocol

The samples were positioned with a sticky wax on metal fins coupled with conical centrifuge tubes

(Falcon, Fisher Scientific, Loughborough, UK) and were submitted to daily simulated rinses. Enamel blocks were exposed to 5 mL of mouth rinses or distilled water twice daily for 14 days under agitation (100 rpm) at room temperature. The exposure time and frequency were performed in accordance with the manufacturer's recommendations. Considering the different mouth rinses used and their commercial presentations, the exposure regimen of 14 days was used as the total consumption time of a bottle of commercial mouth rinse. Information about the groups and mouth rinses used, including manufacturers, pH, and components, are detailed in Table 1. After the stratification of L* values, the samples were randomly divided into five groups (n=10) according to the following treatments:

1. Bleaching with 35% HP and immersion in distilled water for one minute, twice daily for 14 days (C [control])
2. Bleaching with 35% HP and immersion in 225-ppm NaF mouth rinse for one minute, twice daily for 14 days (FM, Colgate Plax Classic)
3. Bleaching with 35% HP and immersion in essential oil–based mouth rinse for 30 seconds, twice daily for 14 days (EM, Listerine Tartar Control)
4. Bleaching with 35% HP and immersion in 1.5% HP-based mouth rinse for two minutes, twice daily for 14 days (HPM, Colgate Plax Whitening)
5. Bleaching with 35% HP and immersion in a mouth rinse containing 2% HP, pyrophosphates, and 225-ppm NaF for one minute, twice daily for 14 days (HPM+P, Colgate Luminous White)

After each rinse treatment, the samples were washed with distilled water for 10 seconds and stored in a remineralizing solution until the next cycle.

Color Measurements

Color reading was performed in an ambient light condition (GTI MiniMatcher MM 1, GTI Graphic Technology Inc, New York, NY, USA) in standardized daylight. The spectral distribution was measured using a reflectance spectrophotometer (CM 700d, Minolta, Osaka, Japan) based on the CIE L*a*b* system. The L* coordinate represents the luminosity (white–black) axis, a* represents the green–red axis, and b* represents the blue–yellow axis. Before the measurements, the spectrophotometer was calibrated using white and black reflectance standards. The analysis was performed at initial time (baseline), 24 hours after dental bleaching, and after use of mouth rinses for 14 days. The color change was calculated using the following equation: $\Delta E = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$.

Surface Roughness

The enamel roughness (Ra) was analyzed using a profilometer tester (Mitutoyo Surfitest 211, São Paulo, Brazil) at three time frames: initial time (baseline), 24 hours after dental bleaching, and after mouth rinse exposure. Three different equidistant scans of 1.25 mm each were measured on the surface of each sample, with a cutoff of 0.25 mm, a load of 5 N, and a velocity of 0.1 mm/s.

Cross-Sectional Microhardness Analysis

The enamel cross-sectional microhardness (CSMH) was analyzed at the end using a Future-Tech FM-ARS microhardness tester (Future-Tech Corp, Tokyo, Japan) with a Knoop diamond under a 50-g load for five seconds. For the CSMH tests, the samples were longitudinally sectioned through the center and embedded in acrylic resin. The exposed area was gradually polished, as previously described. Three rows of five indentations were made in the central area of the slab, the measurements occurring at 10, 25, 50, 75, and 100 μm from the enamel surface. The mean values at all three measuring points at each distance were then determined. Due to the destructive characteristic of the analysis and impossibility of evaluating the baseline values, an unbleached enamel group (n=10) was standardized in order to compare the values found in the experimental groups with intact enamel under the same conditions as those established by the study design.

Scanning Electron Microscopy (SEM)

Three representative samples of groups were randomly selected and subjected to vacuum in a sputter coater (SCD 050 Sputter Coater, Balzers, Liechtenstein) to deposit a thin layer of gold, in order to increase the surface reflectance. Then images of representative areas of the specimens were obtained at 4000× using a scanning electron microscope (JSM 5600LV, JEOL, Tokyo, Japan).

Statistical Analysis

After exploratory analysis using the SAS software (Release 9.1, 2003, SAS Institute Inc, Cary, NC, USA), the data were submitted to one-way analysis of variance (ANOVA) (ΔE), repeated measures ANOVA (Ra), and split-plot ANOVA (CSMH), followed by the Tukey test, at a 5% level of significance.

Table 2: *Mean (SD) for Results of L*, a*, and b* Coordinates Based on Treatment Groups (n=10)*[a]

	Baseline	After Dental Bleaching	After Mouth Rinse
L*			
Control	82.87 (1.80) Ba	85.62 (0.76) Aa	85.38 (0.89) Aa
FM	83.23 (1.62) Ba	86.99 (0.83) Aa	84.44 (1.78) Aa
EM	82.17 (2.45) Ba	85.34 (2.85) Aa	85.60 (0.91) Aa
HPM	83.93 (2.13) Ba	86.86 (1.23) Aa	86.46 (0.65) Aa
HPM+P	81.30 (1.17) Ba	85.93 (1.17) Aa	86.03 (1.03) Aa
a*			
Control	−0.30 (0.50) Aa	−0.71 (0.26) Ba	−0.65 (0.39) Ba
FM	−0.51 (0.38) Aa	−0.60 (0.24) Aa	−0.19 (0.76) Aa
EM	−0.34 (0.37) Aa	−0.49 (0.37) ABa	−0.75 (0.33) Ba
HPM	−0.18 (0.38) Aa	−0.55 (0.31) Ba	−0.96 (0.19) Ca
HPM+P	−0.39 (0.31) Aa	−0.52 (0.27) Ba	−0.97 (0.18) Ca
b*			
Control	10.72 (1.87) Aa	6.20 (1.93) Ba	5.78 (1.80) Ba
FM	9.77 (2.21) Aa	5.08 (1.90) Ba	7.26 (3.70) Ba
EM	9.58 (2.69) Aa	5.26 (1.14) Ba	5.53 (1.16) Ba
HPM	8.70 (3.12) Aa	5.23 (1.59) Ba	3.85 (0.82) Ba
HPM+P	8.83 (2.57) Aa	4.95 (1.38) Ba	5.37 (1.66) Ba

Abbreviations: Rinses based on distilled water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters (upper in horizontal and lower in vertical) are different (p<0.05).

The L*, a*, and b* data were analyzed by generalized linear models (Proc Genmod model) for repeated measures data (α=0.05). Considering the ΔE values, a power calculation was performed with the following parameters: a power value of 0.80, significance level of 0.05, standard deviation = 1.00, and the values proposed by Alghazali and others[21] for the relevant difference between the groups, being 1.9 ΔE units for assessment of perceptibility and 4.2 ΔE units for clinical acceptability of color changes. In this condition, the statistical analysis showed that a minimum sample size of seven would be necessary considering the 1.9 ΔE units or four considering the 4.2 ΔE units. Thus, with a margin of safety, the experiment was conducted with n = 10.

RESULTS

The L*, a*, and b* results are presented in Table 2. For the L* results, the statistical analysis demonstrated an effect of the time factor ($p<0.001$) and an absence of effect of the mouth rinse factor ($p=0.2345$) or interaction of the factors ($p=0.1209$). For the b* values, an effect of the time factor was found ($p<0.001$) and no effect of the mouth rinse factor ($p=0.1363$) or interaction of the factors ($p=0.2427$). For a* data, although no effect was demonstrated for the mouth rinse factor ($p=0.42$), an effect of the time factor ($p<0.001$) and interaction with the mouth rise factor ($p=0.0127$) was statistically significant. Concerning Table 2, for baseline data, there was no statistical difference among the groups. After dental bleaching, the L*, a*, and b* values differed statistically from the baseline values, with increasing L* values and decreasing b* values. Moreover, the groups did not differ statistically for any coordinate studied after the dental bleaching. For the a* values, the EM group did not differ statistically between the times, and the L* and b* values were not different from the other bleached groups. For L* and b* results after mouth rinse exposure, there was no statistical difference among the rinse groups and the control, nor was there a difference compared to the previous time. For the a* results, although no difference among groups was found, a decrease in a* values was statically demonstrated in groups exposed to HP-based mouth rinses (HPM and HPM+P).

Table 3: *Mean (SD) for ΔE Values Based on Treatment Groups (n=10)*[a]

	Baseline vs Dental Bleaching	Dental Bleaching vs Mouth Rinse	Baseline vs Mouth Rinse
Control	5.54 (1.15) a	0.88 (0.40) a	5.79 (0.32) ab
FM	5.59 (0.60) a	3.33 (2.58) a	4.18 (1.55) b
EM	6.27 (1.49) a	1.34 (1.94) a	5.85 (0.58) ab
HPM	5.06 (1.97) a	1.92 (0.98) a	6.28 (1.54) ab
HPM+P	6.56 (1.94) a	1.08 (0.32) a	6.63 (0.96) a

Abbreviations: Rinses based on distilled water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters in vertical are different (p<0.05).

Considering the ΔE values (Table 3) when comparing baseline vs dental bleaching or dental bleaching vs mouth rinse treatment, no difference was shown for all groups, and the groups were not statistically different compared to the control or among them ($p=0.1983$). In assessment of baseline vs mouth rinse exposure, the groups were not different compared to the control ($p=0.0846$); however, FM differed statistically from HPM+P ($p=0.0181$).

Based on the roughness values (Table 4), the initial results of groups were not statistically different. After dental bleaching, a slight increase of Ra values was found in all groups compared to

Table 4: *Mean (SD) for Ra Values (Roughness) Based on Treatment Groups (n=10)*[a]

	Baseline	After Dental Bleaching	After Mouth Rinse
Control	0.13 (0.01) Ba	0.18 (0.02) Aa	0.13 (0.01) Bc
FM	0.13 (0.01) Ba	0.18 (0.02) Aa	0.15 (0.01) Bbc
EM	0.14 (0.01) Ca	0.18 (0.02) Ba	0.21 (0.02) Aa
HPM	0.13 (0.01) Ba	0.18 (0.02) Aa	0.16 (0.02) Ab
HPM+P	0.13 (0.01) Ba	0.18 (0.02) Aa	0.13 (0.01) Bc

Abbreviations: Rinses based on distilled water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters (upper in horizontal and lower in vertical) are different (p<0.05).

baseline values ($p<0.001$), with no difference between groups. In terms of effects on roughness after the mouth rinse application protocol, the control, FM, and HPM+P groups reestablished the baseline values of Ra, which statistically differed from the means of the previous time ($p<0.001$), referring to the values found 24 hours after dental bleaching. Distinct events were observed in the groups exposed to HP mouth rinses: HPM+P showed lower Ra values, statistically differing from the HPM group ($p<0.001$). HPM presented Ra values similar to the previous time and statistically different from the baseline values ($p<0.001$). The blocks treated with EM had the highest increase of Ra, which statistically differed from all other groups ($p<0.01$) or the Ra values in other frames ($p<0.001$).

With regard to cross-sectional microhardness (Table 5), the results showed that the bleached control did not demonstrate a statistical difference from unbleached enamel at 10 and 25 μm. Therefore, the reestablishment of CSMH values was not enabled at all depths after 14 days, being statistically different at 50, 75, and 100 μm from reference values of unbleached enamel ($p<0.001$). There were distinct effects between the rinse groups and the control groups at all depths. The HPM+P group did not differ statistically from the unbleached enamel at depths of 10 and 25 μm, and the FM group did not differ statistically at 25 μm. The HPM+P group did not differ statistically from the bleached control at all depths, and comparing the values of the FM group to the bleached control revealed a statistically significant difference only at 10 μm ($p<0.001$), similar to the results found at depths of 25, 50, 75, and 100 μm. The EM and HPM groups were statistically different from the bleached or unbleached control at all depths ($p<0.001$). In particular, HPM presented lower values of CSMH at all depths. Finally, no statistical differences were found in the evaluation of the CSMH values at the different depths within the group itself.

The SEM images collected are presented in Figure 1. The unbleached enamel (Figure 1A) demonstrated a smooth, regular, and uniform surface. After 24 hours of bleaching (Figure 1B), the enamel surface presented evidence of a slight demineralization process associated with mineral loss, demonstrating a loss of interprismatic substance and an increase in porosity. However, after 14 days in the remineralizing solution, the enamel (Figure 1C) showed mineral recovery and a surface very similar to that verified in the unbleached enamel (Figure 1A). The FM (Figure 1D) and HPM+F (Figure 1G) mouth rinses promoted an enamel surface very similar to unbleached control (Figure 1A) or enamel after 14 days of whitening (Figure 1C) despite rare signs of the demineralizing event that were found in the FM group, as shown in Figure 1D. In fact, distinct severity of such events could be observed throughout the enamel surface in mouth rinse groups as the morphologic changes became much more pronounced in the EM (Figure 1E) and HPM (Figure 1F) groups. The bleached enamel exposed to EM or HPM (Figure 1E,F,

Table 5: *Mean (SD) for Cross-Sectional Microhardness Values Based on Treatment Groups (n=10)*[a]

	10 μm	25 μm	50 μm	75 μm	100 μm
Unbleached enamel	359.2 (28.6) Aa	356.1 (30.0) Aa	372.9 (28.4) Aa	374.6 (30.3) Aa	371.6 (29.6) Aa
Dental bleaching (35% HP) +					
Distilled water	324.8 (30.0) Aa	325.7 (24.9) Aa	321.4 (28.6) Ab	325.4 (31.8) Ab	315.2 (27.2) Ab
FM	258.6 (34.1) Ab	309.7 (22.6) Aa	301.8 (31.6) Ab	297.7 (30.5) Abc	299.0 (32.3) Ab
EM	241.1 (28.4) Ab	258.7 (24.7) Ab	246.9 (28.8) Ac	260.7 (29.8) Ac	248.7 (29.8) Ac
HPM	172.5 (32.4) Ac	181.1 (34.8) Ac	187.7 (32.5) Ad	180.3 (30.5) Ad	183.2 (23.9) Ad
HPM+P	313.0 (14.1) Aa	316.1 (27.0) Aa	306.4 (20.6) Ab	321.1 (11.5) Ab	321.5 (16.2) Ab

Abbreviations: Rinses based on water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters (upper in horizontal and lower in vertical) are different (p<0.05).

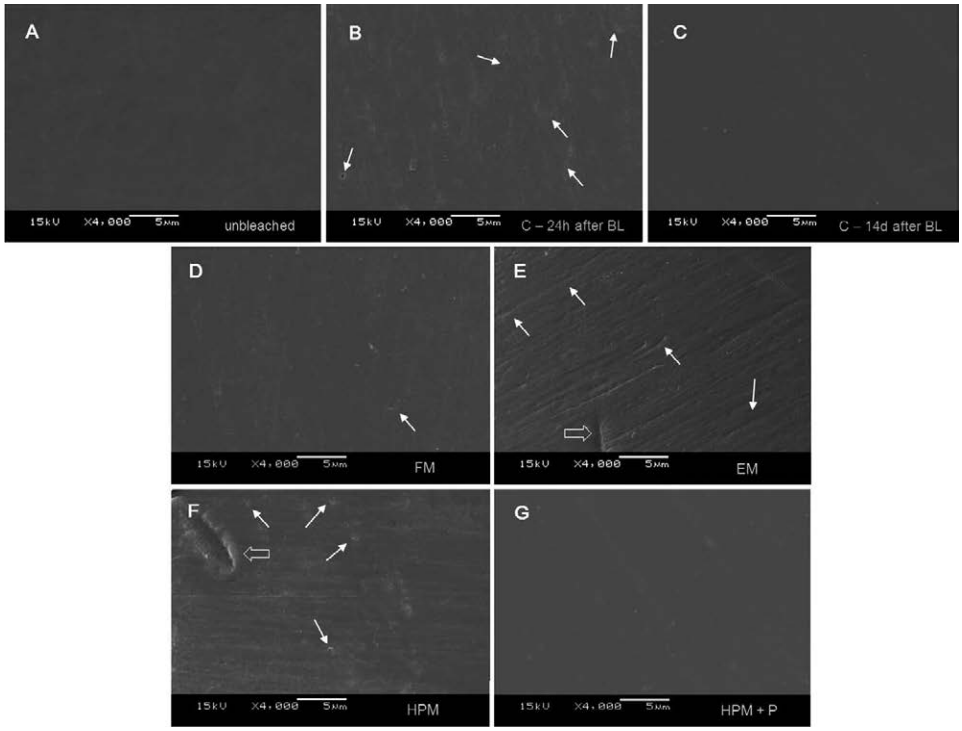

Figure 1. *Representative scanning electron microscopic images (4000×) of the samples. (A): Unbleached enamel. (B) Bleached enamel after 24 hours. (C): Bleached enamel after 14 days and exposed to distilled water (control). Bleached enamel with 35% hydrogen peroxide and submitted to mouth rinses.: (D): 225-ppm NaF (FM, Colgate Plax Classic). (E): Essential oil (EM, Listerine Tartar Control). (F): 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening). (G): 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White). The morphological alterations found on the enamel surface of groups are represented in the images in B, D, E, and F; the fine arrows represent areas with pores or surface irregularities, and thick arrows indicate depressions of enamel.*

respectively) presented pores or superficial irregularities with intermittent depressions. Linear markings associated with SiC polishing can be visualized in the images (Figure 1A-G); however, only the results associated with morphological changes, such as loss of interprismatic substance, depressions, and porosities, were described, and possible linear markings were not considered or associated with the treatments studied.

DISCUSSION

In the present study, null hypotheses 1 and 3 were rejected because the in-office dental bleaching affected the physical properties of enamel, and the mouth rinse exposure promoted different effects on enamel, modifying the physical properties and mineral recovery of the tooth. However, null hypothesis 2 was accepted because the exposure to mouth rinse after the in-office dental bleaching did not affect the whitening effectiveness of treatment. Dental enamel is the hardest mineralized biological tissue, containing approximately 96% mineral, 3% water, and 1% organic matter by weight. The enamel blocks were obtained from bovine incisors, as they present physical-chemical properties resembling that of human enamel[22] and are considered a practical model for bleaching studies.[23] All groups in this investigation indicated significant change in L* and b* values following dental bleaching. The whitening effectiveness of treatment was evidenced due to the fact that the mean L* values increased while the mean b* values decreased, which represents a lighter and less yellowish color for the tooth. The mean values of total color change (ΔE) after in-office dental bleaching were greater than 4.2 units[21] or 3.3 units,[24,25] the standard values suggested for clinical acceptability of color differences.

On the other hand, the use of mouth rinses did not act directly on the L*, b*, and ΔE values. The HP mouth rinses (HPM and HPM+P), commercially available as whitening mouthwashes (OTC), were not able to promote an improvement of the bleaching effect. These mouth rinses are composed with a low concentration of HP that could diffuse through the dental structure and produce free radicals that lead to successful bleaching;[3,26] however, the effect found in the present study may have been low due to the fact that they stay in contact with the enamel for a short period of time compared with those offered by dentist-guided treatments[27] in addition to a lower concentration of active ingredient.

In relation to colorful mouth rinses, essential oil–containing mouth rinses (EM) are made available as a blue-colored alcohol solution and have been associated with enamel pigmentation after prolonged contact exposure.[17] Despite the evidence that bleached enamel may be more susceptible to staining,[28,29] the EM group did not promote color change

or disrupt the color stability of treatment, possibly because the exposure was performed daily under more real conditions. The FM group, commercially available as a red solution, presented $\Delta E = 3.33$, which could even indicate visually unacceptable discoloration according to the previous investigations;[24,25] however, the color results showed an absence of statistical differences, demonstrating the necessity of more studies to investigate the effects on color of enamel exposed to FM mouth rinse for a longer time. Overall, the use of a colorful mouth rinse after in-office dental bleaching did not affect the efficacy of whitening treatment, corroborating a previous review[30] that concluded that the use or ingestion of products with dyes does not limit the effect of tooth whitening.

According to the results of this present study, the dental bleaching with 35% HP promoted an increase in enamel surface roughness, a slight change in topography visualized by SEM, and a decrease in the cross-sectional microhardness, as previously described.[6,12,31,32] During dental bleaching, a mineral dissolution[8,33,34] could occur, explaining the alterations on enamel properties. These deleterious effects can be attributed to the oxidation of the organic and inorganic components of the tooth by free radicals[3,26] as well as to the acidic pH of the bleaching agent used,[35] which was 4.87 in the current study (Table 1). However, after 14 days, the bleached enamel showed Ra values and enamel surface (SEM) similar to the unbleached enamel as well as no difference from unbleached enamel at 10 and 25 μm in the CSMH analysis. The remineralizing solution was used like artificial saliva to simulate the inorganic composition of human saliva, and this storage produced an environment rich in calcium, phosphorus, and fluoride. This environment promoted enamel remineralization, enabling the mineral recovery of dental substrates and almost completely reversing the demineralization caused by the bleaching agent, with an exception at the depths of 50, 75, and 100 μm.

Saliva[36] and other active agents[10-12] play an essential role in promoting remineralization or decreasing demineralization of teeth submitted to bleaching treatment. The rinses studied did not present additional or beneficial effects to bleaching therapy, and in some cases were able to potentiate an injury to the dental structure. After mouth rinse cycling, the EM (Listerine Tartar Control) induced enamel demineralization, verified through changes on the surface, the highest increase of Ra, and a decrease of microhardness values in depth. These alterations may have occurred due to the low pH of the product associated with the absence of remineralizing agents in an alcoholic vehicle.[18,19,37] Although the impact of this increased roughness promoted by EM in clinical practice is undesired, these results indicate a change in surface topography promoted by a demineralizing event with possible dissolution of hydroxyapatite crystals with slight structural alterations, as shown in Figure 1E.

In addition, the OTC whitening products could cause undesirable local effects, such as sensitivity, oral mucosa irritation, alterations of physical properties of restorative materials, and slight erosion in the tooth structure.[9] The potential abusive use of these self-agents, especially in young patients, could promote potential harmful results.[38] The OTC agents evaluated in the present study exhibited different results in relation to enamel properties; while HPM was damaging to dental structure for all variables studied, the HPM+P presented a surface and subsurface similar to the unbleached enamel. This could be explained by the different pH of these products (Table 1) and the fact that the HPM (Colgate Plax Whitening) does not have any remineralizing agent in its composition and remained in greater contact with the enamel, which happened for two minutes, as the manufacturer indicated for a prebrushing rinse. Furthermore, pyrophosphates and fluoride are incorporated into HPM+P (Colgate Luminous White).

Pyrophosphates are agents with high affinity for hydroxyapatite crystal, interacting with calcium.[39] During the chemical reactivity with enamel, the pyrophosphates reduce the binding capacity of proteins or chromogens, being considered an anticalculus or antistaining agent.[39] Additionally, HPM+P and FM include added fluoride (225-ppm NaF), which is currently used as an agent that promotes remineralization of dental hard tissues and decreases the effects of demineralization.[40] The HPM+P and FM presented neutral pH; however, the presence of low-concentration fluoride during the demineralizing event in rinse solutions or bleaching gel[41] appears to be more important than its use after tooth whitening to remineralize because no evidence of remineralization was found in the FM group, commercially available as a neutral fluoride rinse solution (pH=6.11).

This study was designed to evaluate the effects on enamel of mouth rinse exposure after in-office tooth bleaching. The impact of active agents incorporated into mouth rinses on enamel is very relevant because oral home care products are purchased

and sold cosmetically and, unfortunately, often used without supervision by a dentist. The consumption of oral home care products has spread around the world, and HP- or alcohol-containing mouth rinses need to be extensively investigated, especially with regard to the rational use and safety of these products. However, it is important to note that the *in vitro* studies of solutions at low pH have been shown to exaggerate the erosive/demineralization effect. In the mouth, the mineral dissolution could be lower because these effects on mineral content are decreased by the protective effect of the acquired pellicle and the buffering capacity of human saliva. Nevertheless, controlled *in vitro* studies are necessary and important precursors of *in vivo* studies. Further research is needed to investigate the performance of mouth rinses in *in situ* and *in vivo* models regarding the combination of compounds, wear time, and the association of different oral care products with the dentist-supervised bleaching, whether using at-home or in-office techniques.

CONCLUSIONS

The use of a mouth rinse after in-office dental bleaching did not affect the efficacy of whitening treatment or enhance tooth staining. Additionally, the mouth rinses did not promote additional benefits to treatment, and the 1.5% HP- or essential oil–based rinses impaired mineral reestablishment of enamel, promoting a decrease in bleached enamel properties.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the University of Campinas.

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.

(Accepted 20 February 2018)

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Effect of Active Application on Bond Durability of Universal Adhesives

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Clinical Relevance

Active application may be helpful for enhancing the dentin bond durability of universal adhesives independent of etching mode.

SUMMARY

The purpose of this study was to determine the effect of different adhesive application methods and etching modes on the dentin bond durability of universal adhesives under thermal cycling (TC). All-Bond Universal (Bisco), Adhese Universal (Ivoclar Vivadent), and Scotchbond Universal (3M ESPE) were used as adhesives. In total, 600 bovine teeth with exposed dentin were divided into 12 groups according to the type of adhesive and subjected to the following bonding procedures: 1) etch-and-rinse mode with active application; 2) etch-and-rinse mode with inactive application; 3) self-etch mode with active application; and 4) self-etch mode with inactive application. Bonded specimens were stored in distilled water at 37°C for 24 hours and then subjected to 5000, 10,000, 30,000, or 50,000 TC between 5°C and 55°C before shear bond strength (SBS) testing, creating a division into a total of five different storage conditions. Baseline specimens were stored in distilled water at 37°C for 24 hours. The SBS test was performed at a cross-head speed of 1.0 mm/min. Three-way analysis of variance revealed that all the factors of application mode, adhesive, and thermal cycle period significantly influenced the SBS values ($p<0.001$), regardless of the etching mode. In the baseline groups, all of the tested adhesives with active application had higher SBS values than those with inactive application, regardless of etching mode. In the TC groups, significantly lower SBS values were observed at 50,000 TC with inactive application compared to those with active application, regardless of the etching mode. From the scanning electron microscopy observation of demineralized and deproteinized resin/dentin interfaces, dense resin tags longer than 50 μm were observed in the etch-and-rinse

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DOI: http://doi.org/10.2341/17-384-L

with active application group. On the other hand, the resin tags in self-etch mode were sparse, thin, and much shorter than those in etch-and-rinse mode. Comparing the penetration status of the resin tags with active and inactive application in self-etch mode, the resin tag penetration with inactive application was much lower than that with active application. Active application is effective in enhancing the dentin bond durability of universal adhesives. When using universal adhesives with different etching modes, practitioners should select the optimal etching mode and appropriate application method in accordance with the cavity conditions.

INTRODUCTION

A prepared tooth consists of complex structures with more than one component and unique morphologies. It is essential for restorations to bond effectively to the external and internal walls of a prepared tooth despite the cavity complexity. One of the most significant problems with restorations is that enamel and dentin respond differently to various treatments. Universal adhesives are versatile and capable of sensitive adaptation to a variety of cavity conditions.[1-4] However, work on the best techniques to use with universal adhesive is still at an early stage.

Phosphoric acid etching of dentin before self-etch adhesive application is believed to have a negative impact on bonding.[5-7] The procedure results in an incompletely hybridized region in the vicinity of the resin-dentin interface, which may be a critical factor in the bond degradation of self-etch adhesives.[8-10] Phosphoric acid dissolves the hydroxyapatite (HAp) surrounding the collagen fibrils, causing the chemical bonds between HAp and functional monomers to decline both in quality and quantity.[7,8] Therefore, when using self-etch adhesives, avoiding phosphoric acid pre-etching or selectively etching only the enamel region are recommended.[11,12] In the particular case of enamel bonding when using self-etch adhesives, selective etching of enamel is effective because it creates stronger micromechanical interlocking and increases surface wettability.[13,14] However, it is difficult to precisely etch only the enamel region, particularly with small tooth preparations, complex configurations, or proximal surface area. Hence, concerns remain that the phosphoric acid agent may attack the dentin region of the cavity.

Universal adhesives have distinctive characteristics compared to the previous generations of adhesive systems. These adhesives can be used with different types of substrates, such as enamel, dentin, silica-based glass ceramics, zirconia ceramics, and metal alloys.[15-18] Therefore, universal adhesives can be used not only for direct resin composite restorations but also for indirect restorations. In addition, universal adhesives are expected to help simplify the bonding procedure for patch restorations used to repair aged restorations with flaws on the surface. Another important characteristic of universal adhesives is that they can be used with either etch-and-rinse or self-etch approaches.[2,3] Universal adhesives enable the practitioner to select the optimal etching approach based on the cavity conditions, including depth, size, location, and the proportion of enamel and dentin. In general, universal adhesives may be classified as single-step self-etch adhesives as a result of their usage and broadly similar composition to that of single-step self-etch adhesives.

Previous studies[2,3,19,20] showed that although the enamel and dentin bond strengths of universal adhesives were not higher and indeed were often lower than those of two-step self-etch adhesives, they were equal to or greater than those of single-step self-etch adhesives. However, there is concern that the technology of universal adhesives may not offer a genuine advantage when compared to previous generations of single-step self-etch adhesives.[15,21] For example, when considering the dentin bonding of universal adhesives in etch-and-rinse mode, concerns are raised that the chemical bonds between HAp and functional monomers may be reduced as a result of lack of HAp, as is the case with single-step self-etch adhesives.

To enhance the bonding effectiveness of universal adhesives, an active adhesive application technique is recommended.[1,4] Active application of adhesives may lead to micromechanical interactions with the underlying mineralized tissue and penetration into the tooth substrate.[22] Stirring the adhesives can also accelerate chemical reactions between HAp and functional monomers.[23] That is, active application may mobilize a greater number of H^+ ions to react with mineral components.[4] Imai and others[4] evaluated the influence of adhesive application methods on enamel bond strength and surface free energy using four commercially available universal adhesives under different etching modes and concluded that although active application was effective in self-etch mode, it had a negative impact on enamel bond strength in etch-and-rinse mode. Although active application may enhance enamel bonding performance with the self-etch approach, little information is available on the effect of active application on the

Table 1: *Materials Used in the Study*

Code	Adhesive (Lot No.)	Main Components	Classification According to pH	Manufacturer
AB	All-Bond Universal (1300008503)	MDP phosphate monomer, Bis-GMA, HEMA, ethanol, water, initiators	Ultra-mild (pH=3.2)[a]	Bisco Schaumburg, IL USA
AU	Adhese Universal (U49302)	MDP, Bis-GMA, HEMA, MCAP D3MA, ethanol, water, initiator, silicon dioxide, stabilizers	Ultra-mild (pH=2.5-3.0)[a]	Ivoclar Vivadent Schaan, Lichtenstein
SU	Scotchbond Universal (41256)	MDP phosphate monomer, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	Ultra-mild (pH=2.7)[a]	3M ESPE Dental Products St Paul, MN, USA
	Etching agent Ultra-Etch (G017)	35% phosphoric acid		Ultradent Products South Jordan, UT, USA
	Resin composite Clearfil AP-X (N416713)	Bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, CQ, pigments, others		Kuraray Noritake Dental Tokyo, Japan

Abbreviations: Bis-GMA, 2,2-bis [4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane; CQ, dl*-camphorquinone; D3MA, decandiol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MCAP, methacrylated carboxylic acid polymer; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethyleneglycol dimethacrylate;*
[a] *References 1, 19.*

long-term dentin bond performance of universal adhesives in different conditions.

The purpose of the present study was to determine the effect of active application on bovine dentin bond durability when using different etching modes with universal adhesives by measuring the bond strength after thermal cycling, coupled with morphological observations of adherent surfaces and the resin-dentin interfaces. The null hypothesis was that active application does not affect dentin bond durability, regardless of the etching mode used.

METHODS AND MATERIALS

Study Materials

The materials used in this study are shown in Table 1. The three universal adhesives used were 1) All Bond Universal (AB; Bisco, Schaumburg, IL, USA), 2) Adhese Universal (AU; Ivoclar Vivadent, Schaan, Liechtenstein), and 3) Scotchbond Universal (SU; 3M ESPE, St Paul, MN, USA). These products are recommended for use with active application. The phosphoric acid pre-etching agent used was Ultra-Etch (Ultradent Products, South Jordan, UT, USA). Clearfil AP-X (Kuraray Noritake Dental, Tokyo, Japan) was used as a restorative material for bonding to dentin. A visible light-curing unit (Optilux 501, sds Kerr, Danbury, CT, USA) was used, and the light irradiance (average 600 mW/cm^2) of the curing unit was checked using a dental radiometer (Model 100, Demetoron, Lincoln, NE, USA). During the course of this experiment, we checked the light irradiance of the quartz-tungsten halogen light-curing unit before use in each experimental group. When the light irradiance of the curing unit measured below 600 mW/cm^2, we changed to a new halogen lamp and confirmed its irradiance.

Specimen Preparation

Extracted mandibular bovine incisors stored frozen for up to two weeks were used. Approximately two-thirds of the apical root structure of each tooth was removed using a diamond-impregnated disc in a low-speed saw (IsoMet 1000, Precision Sectioning Saw, Buehler, Lake Bluff, IL, USA). The labial surface of each tooth was ground with wet #240-grit silicon-carbide (SiC) paper (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan) to create a flat dentin surface. Each tooth was mounted in self-curing acrylic resin (Tray Resin II, Shofu Inc, Kyoto, Japan) to expose the flattened area. Dentin adherent surfaces were polished using a water coolant and a series of SiC polishing papers, ending with 320-grit SiC paper (Fuji Star Type DDC). This grit was chosen for consistency with the ISO 29022[24] Standard for the shear bond strength (SBS) tests, and with clinical conditions.

Thermal Cycling (TC) and SBS Tests

The prepared dentin adherent surfaces were treated in accordance with the experimental protocol for the bonding procedures (Table 2). Six hundred specimens in total were divided into 12 groups according to the type of adhesive and subjected to the following surface treatments (10 specimens for each group): 1) etch-and-rinse mode (phosphoric acid applied for 15 seconds prior to application of the adhesives) with active application, 2) etch-and-rinse mode with inactive application, 3) self-etch mode (without phosphoric acid etching) with active application,

Table 2: *Application Protocol for Pre-etching and Self-etching Adhesives*

Method		**Pre-etching Protocol**
Etch-and-rinse		Dentin surface was conditioned with phosphoric acid for 15 s. Conditioned surface was rinsed with water for 15 s (three-way dental syringe) and air-dried.
Self-etch		Phosphoric acid pre-etching was not performed.
Code	**Application Method**	**Adhesive Application Protocol**
AB	Active application	Adhesive was applied to dentin surface (not desiccated) with rubbing action for 10-15 s per coat. No light cure between coats. Gentle stream of air applied over the liquid for at least 10 s. Light irradiation performed for 10 s.
	Inactive application	Adhesive was applied to dentin surface (not desiccated) without rubbing action for 10-15 s per coat. No light cure between coats. Gentle stream of air applied over the liquid for at least 10 s. Light irradiation performed for 10 s.
AU	Active application	Adhesive was applied to the dentin surface with rubbing action for 20 s, followed by application of medium air pressure for 5 s. Light irradiation performed for 10 s.
	Inactive application	Adhesive was applied to the dentin surface with rubbing action for 20 s, followed by application of medium air pressure for 5 s. Light irradiation performed for 10 s.
SU	Active application	Adhesive was applied to the dentin surface using rubbing action for 20 s, followed by application of medium air pressure for 5 s. Adhesive light cured for 10 s.
	Inactive application	Adhesive was applied to the dentin surface without rubbing action for 20 s, followed by application of medium air pressure for 5 s. Adhesive light cured for 10 s.

and 4) self-etch mode with inactive application. The adhesive agents were applied using a microbrush either with (active) or without (inactive) a rubbing motion. In the active application group, the adhesive was rubbed for the duration indicated by the manufacturer, whereas in the inactive application group, the adhesive was allowed to stand for the same period of time. An Ultradent bonding assembly (Ultradent Products) was used in this study. Following adhesive application to the dentin adherent surface, bonded resin composite cylinders were built on dentin surfaces with plastic molds (Bonding Mold Insert, 2.4 mm in internal diameter, approximately 2.5 mm in height, Ultradent Products) in the fixture (Bonding Clamp, Ultradent Products) against the dentin surfaces. The resin composite was condensed into the mold, and light irradiation was applied for 30 seconds with a curing unit. The bonded specimens were stored in distilled water at 37°C for 24 hours and then treated with 5000, 10,000, 30,000, or 50,000 thermal cycles (TC) between 5°C and 55°C with a dwell time of 30 seconds, creating a division into a total of five different storage conditions. Baseline specimens were stored in distilled water at 37°C for 24 hours before the SBS tests (baseline group). The SBS of the three universal adhesives to dentin was measured using the notched-edge SBS test, as described in ISO 29022.[24] The bonded specimens were loaded to failure at 1.0 mm/min with a shearing fixture (Test Base Clamp, Ultradent Products) using a universal testing machine (Type 5500R, Instron Corp, Canton, MA, USA). SBS values (MPa) were calculated by dividing the peak load at failure by the bonded surface area. After testing, the bonding sites on the tooth surfaces and the resin composite cylinders were observed under an optical microscope (SZH-131, Olympus, Tokyo, Japan) at a magnification of 10× to determine the bond failure mode. Based on the percentage of substrate area (adhesive–resin composite–dentin) observed in the debonded resin composites and tooth bonding sites, bond failure was classified into 1) adhesive failure, 2) cohesive failure in the composite, 3) cohesive failure in the dentin, or 4) mixed failure, defined as partially adhesive and partially cohesive.

Scanning Electron Microscopy (SEM) Observation

Representative treated dentin surfaces, resin-dentin interfaces, and debonded fracture sites were observed using SEM (ERA-8800FE, Elionix, Tokyo, Japan). Dentin surfaces were first treated in accordance with the experimental protocol for bonding procedures, then rinsed with acetone and water. For ultrastructural morphological observations of the resin-dentin interfaces to determine the penetration of the adhesives, the bonded specimens stored in 37°C distilled water for 24 hours were embedded in epoxy resin and longitudinally sectioned using a low-speed saw (IsoMet 1000). The sectioned surfaces were polished to a high gloss with SiC papers (Fuji Star Type DDC) followed by diamond pastes down to a particle size of 0.25 μm (DP-Paste, Struers, Ballerup, Denmark). After ultrasonic cleaning for three min-

Table 3: *Influence of Thermal Cycling (TC) on Shear Bond Strength (SBS)— Etch-and-rinse Mode*[a]

	24-h	5000 TC	10,000 TC	30,000 TC	50,000 TC
AB					
Active	41.5 (6.4)^{aA}	41.9 (4.8)^{aA}	38.6 (3.7)^{bA}	39.6 (3.5)^{bA}	39.8 (5.0)^{abA}
Inactive	36.2 (4.6)^{abA}	38.6 (4.6)^{abA}	33.5 (3.9)^{bcAB}	35.1 (5.5)^{bAB}	32.1 (3.5)^{cB}
AU					
Active	32.7 (6.6)^{bA}	37.0 (4.7)^{abA}	35.9 (7.4)^{bcA}	36.6 (6.6)^{bA}	40.0 (4.0)^{aA}
Inactive	29.3 (5.3)^{bA}	30.6 (4.8)^{cA}	30.4 (5.2)^{cA}	27.8 (5.1)^{cA}	15.3 (5.0)^{dB}
SU					
Active	35.2 (4.3)^{abC}	41.0 (3.7)^{aB}	45.3 (2.7)^{aA}	47.5 (1.8)^{aA}	43.8 (1.8)^{aAB}
Inactive	33.5 (1.9)^{bB}	34.0 (5.6)^{bB}	40.2 (2.6)^{abA}	37.6 (3.6)^{bAB}	34.6 (3.9)^{bcB}

[a] *Same lowercase letters in vertical columns indicate no significant difference. Same capital letters in horizontal rows indicate no significant difference;* $p > 0.05$.

utes, the polished surface was etched with HCl solution (6 mol/L) for 25 seconds and deproteinized by immersion in 6% NaOCl solution for three minutes. The debonded specimens from each storage condition were prepared directly for SEM. All SEM specimens were dehydrated in ascending grades of *tert*-butyl alcohol (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 (Model ID-3, Elionix) for 30 minutes. The resin-dentin interfaces of the specimens were subjected to argon-ion beam etching (EIS-200ER, Elionix) for 20 seconds using an ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm^2) directed perpendicular to the polished surfaces. Finally, all SEM specimens were coated with a thin film of gold in a vacuum evaporator (Quick Coater, Type SC-701, Sanyu Denshi, Tokyo, Japan). Observations were performed under SEM at an operating voltage of 10 kV.

Statistical Analysis

A statistical power analysis indicated that at least nine samples were necessary for effective measurement of bond strength. Therefore, this experiment was initially performed with sample sizes of 10. After gathering the data, post hoc power tests were performed, and these tests indicated that the sample size was adequate. Because of their homogeneity of variance (Bartlett test) and normal distribution (Kolmogorov-Smirnov test), the data obtained from each adhesive were subjected to analysis of variance (ANOVA) followed by Tukey honestly significant difference test at a significance level of 0.05. Three-way ANOVA was used for statistical analysis of the etch-and-rinse and self-etch mode SBS data separately. All statistical analyses were performed using the Sigma Plot software (ver 11.0; SPSS Inc, Chicago, IL, USA).

RESULTS

SBS Measurements

Three-way ANOVAs for SBS in etch-and-rinse mode revealed that all the factors evaluated significantly influenced the SBS values ($p<0.001$), and the three-way interactions between the evaluated factors and all the interactions were significant ($p<0.005$). Three-way ANOVA for the SBS values in self-etch mode revealed that all the factors significantly influenced the SBS ($p<0.001$), similar to the results for the etch-and-rinse mode. Although the three-way interaction between the factors and the interactions between the adhesives and thermal cycles significantly influenced the SBS values ($p<0.05$), other pairwise interactions were not significant (application method vs adhesive; $p=0.302$, and application method vs thermal cycle: $p=0.851$).

The SBS values in etch-and-rinse mode under different numbers of TC are shown in Table 3. For all the adhesives in the baseline groups (24-hour water storage), no significant differences were observed in the SBS values between the active and inactive application groups. For the TC groups, all the adhesives in the inactive application group showed lower SBS values as the number of thermal cycles increased, and significant differences were found between the active and inactive application groups at 50,000 TC cycles. On the other hand, all of the universal adhesives in the active application group were observed to have reliable SBS values, in that there were no significant SBS reductions in the 50,000 TC groups when compared to those of the baseline groups.

The SBS results in self-etch mode under different numbers of TC are shown in Table 4. For the baseline groups, similar to the etch-and-rinse mode, all the adhesives showed higher SBS values with the

Table 4: *Influence of Thermal Cycling (TC) on Shear Bond Strength (SBS)—Self-etch Mode*[a]

	24-h	5000 TC	10,000 TC	30,000 TC	50,000 TC
AB					
Active	40.2 (2.0)^{aA}	37.8 (3.0)^{abA}	39.9 (3.9)^{abA}	41.0 (2.7)^{aA}	41.7 (2.2)^{aA}
Inactive	37.9 (2.0)^{abA}	32.9 (3.2)^{cB}	33.3 (2.9)^{cB}	35.7 (3.8)^{bcAB}	35.5 (4.3)^{bcAB}
AU					
Active	34.1 (4.4)^{bC}	41.8 (5.1)^{aAB}	43.2 (3.3)^{aA}	37.0 (2.8)^{bcBC}	31.3 (3.3)^{cdC}
Inactive	28.2 (4.1)^{cB}	38.0 (2.6)^{abA}	37.2 (2.1)^{bcA}	34.5 (3.4)^{cdA}	29.9 (2.6)^{dB}
SU					
Active	37.7 (5.7)^{abA}	38.2 (3.5)^{abA}	40.2 (2.6)^{abA}	38.8 (2.5)^{abA}	39.2(2.2)^{abA}
Inactive	34.6 (3.7)^{bAB}	34.9 (4.8)^{bcAB}	38.1 (2.8)^{bA}	32.8 (1.5)^{dB}	33.4 (4.6)^{cdB}

[a] *Same lowercase letters in vertical columns indicate no significant difference. Same capital letters in horizontal rows indicate no significant difference;* $p > 0.05$.

active application method, and the difference was significant for AU. For the TC groups, all the adhesives in the active application group showed higher SBS values than with inactive application at all TC conditions. However, neither application method showed significant differences in SBS between the 50,000 TC and the baseline groups. When observing the SBS values under different TC conditions, all the adhesives showed similar, but not identical, patterns of change. The SBS value for AU in etch-and-rinse mode at 50,000 TCs was markedly lower than that for the other adhesives (15.3 compared to 30 to 40).

Failure Mode Analysis

The frequencies of different failure modes are shown in Figures 1 and 2. Adhesive failure was most commonly observed in all debonded baseline specimens, regardless of the etching mode, type of adhesive, or application method. When TC was applied, although mixed failure and cohesive failure in dentin were observed frequently for all the adhesives at 5000 TC, the adhesive failure increased with higher TC numbers. In particular, this trend was obvious in self-etch mode with active application, regardless of the type of adhesive. When comparing the failure pattern between active and inactive application, adhesive failure was more common in the inactive application group, regardless of the TC condition.

SEM Observations

Representative SEM images of the treated dentin surfaces are shown Figures 3 and 4. In etch-and-rinse mode, complete removal of the smear layer, smear plugs, and open dentin tubules was clearly observed for all the adhesives tested, regardless of the application method (Figures 3 and 4). For AU, greater amounts of deposit on the etched surface were observed in the active application group than in the inactive group (Figure 4). On the other hand, for the specimens in self-etch mode, the smear layer and the scratch marks caused by the SiC paper were clearly visible regardless of the adhesive and application method used. However, the smear layer and smear plugs were dissolved in some locations with the active application method (Figures 3 and 4), in contrast to the inactive method (Figures 3and 4).

Representative SEM images of demineralized and deproteinized resin/dentin interfaces are shown in Figures 5 and 6. For all the adhesives, dense resin tags longer than 50 μm were observed in the etch-and-rinse with active application group. Although resin tags with a similar length were observed in both the active and inactive application groups for AB, the resin tags for SU and AU in the inactive application group were obviously shorter than those in the active application group. A hybrid layer of approximately 1 to 2 μm was observed in etch-and-rinse mode, regardless of the adhesive or application method used. On the other hand, the resin tags in self-etch mode were sparse, thin, and much shorter than those in etch-and-rinse mode, and a hybrid layer was not observed, regardless of the adhesive or application method used. Comparing the penetration status of the resin tags with active and inactive application in self-etch mode, the resin tag penetration with inactive application was much lower than that with active application.

Representative SEM images of the failure sites after the bond strength test are shown in Figures 7 and 8. The appearance of the failure patterns was dependent on the etching mode, application method, and storage condition. For the baseline groups in etch-and-rinse mode, the failure sites primarily showed detachment at the adhesive-dentin interface,

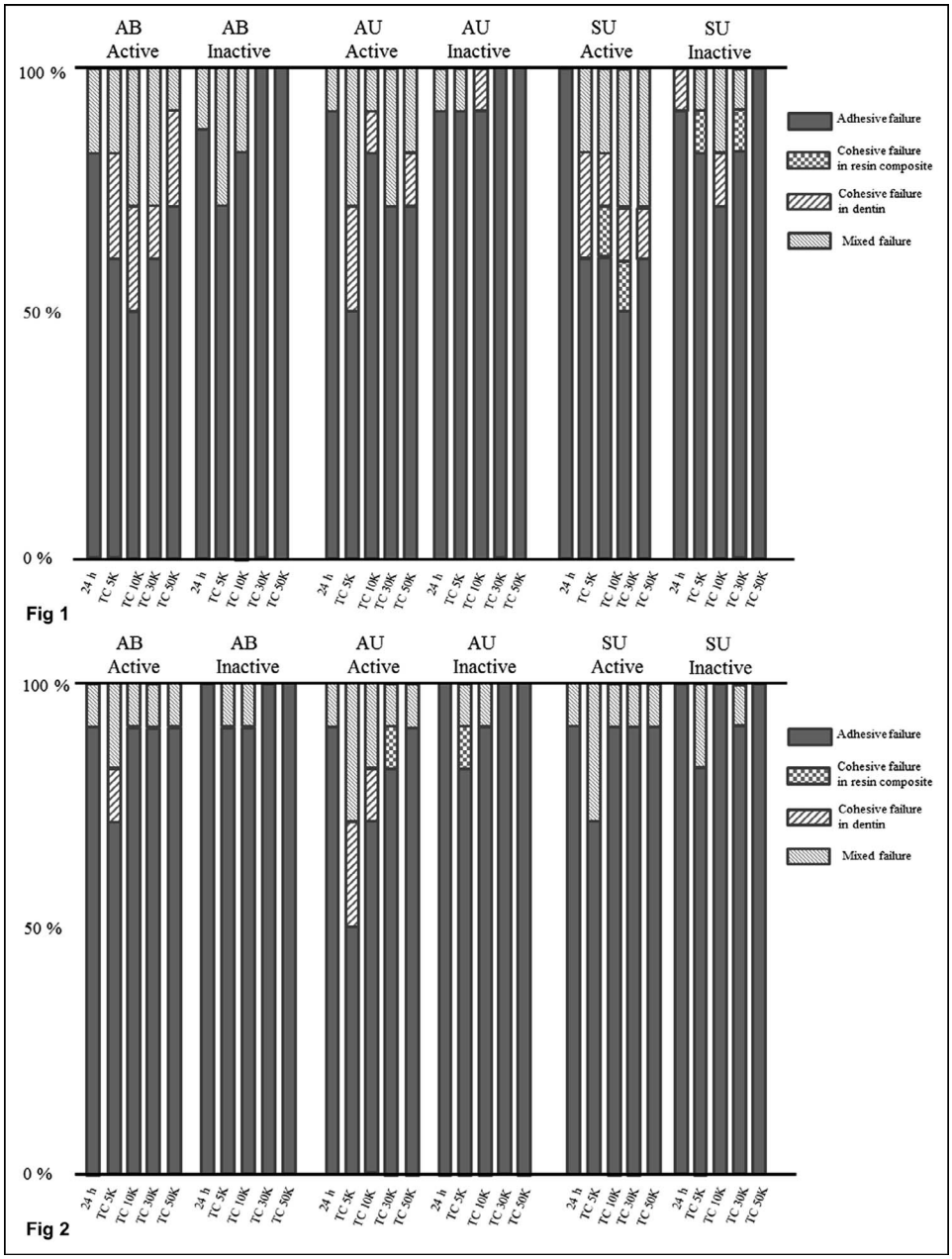


Figure 1. *The frequencies of different failure modes in etch-and-rinse mode.*

Figure 2. *The frequencies of different failure modes in self-etch mode.*

and evidence of resin tags was clearly visible, regardless of the application method (Figure 7). For the 50,000 TC specimens in etch-and-rinse mode, although evidence of resin tags was observed with both application methods, they were less clear than in the baseline specimens (Figure 7). In particular, a flat and smooth failure site was observed with inactive application (Figure 7). For debonded specimens in self-etch mode, the failure site included detachment at both the adhesive-dentin and adhesive-resin composite interfaces at lower magnification, regardless of the storage condition (Figure 8). At higher magnification, although evidence of resin tags was barely observable with both application methods in the baseline groups (Figure 8), it was difficult to observe any evidence of resin tags after thermal cycling (Figure 8).

DISCUSSION

In this study, no significant differences in dentin SBS were found between active and inactive application in the baseline groups, with the exception of AB in self-etch mode. However, all the adhesives showed significantly higher dentin SBS values in the active application groups than in the inactive

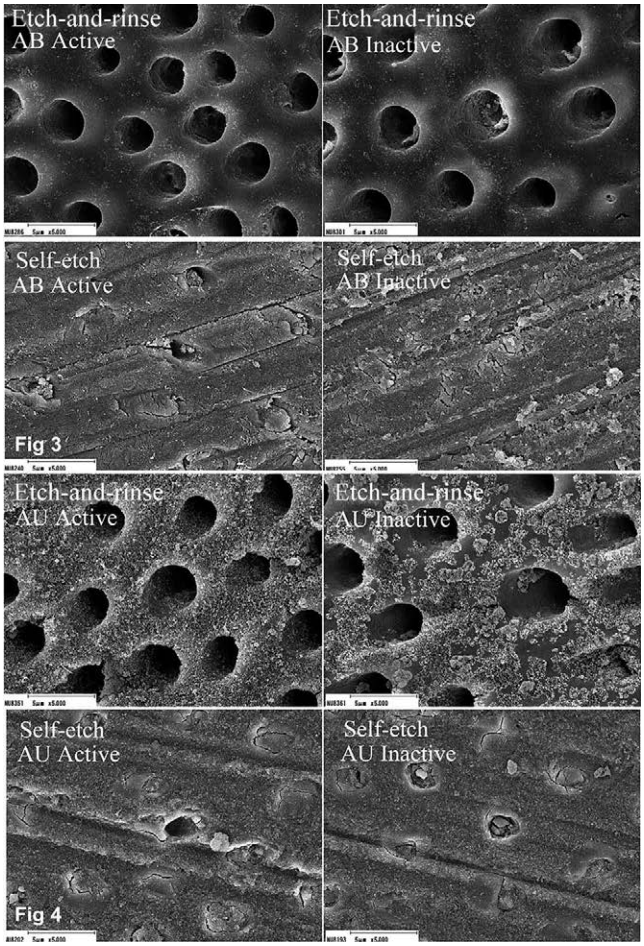


Figure 3. *Representative SEM images of treated dentin surfaces (AB). Upper left image: AB in etch-and-rinse mode with active application method (,000×). Upper right image: AB in etch-and-rinse mode with inactive application method (5000×). Lower left image: AB in self-etch mode with active application method (5000×). Lower right image: AB in self-etch mode with inactive application method (5000×).*

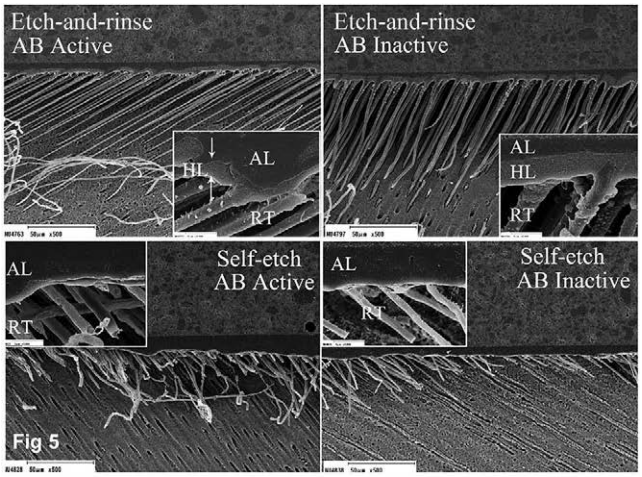
Figure 4. *Representative SEM images of treated dentin surfaces (AU). Upper left image: AU in etch-and-rinse mode with active application method (,000×). Upper right image: AU in etch-and-rinse mode with inactive application method (5000×). Lower left image: AU in self-etch mode with active application method (5000×). Lower right image: AU in self-etch mode with inactive application method (5000×).*

application groups at 50,000 TC, regardless of the etching mode used. Therefore, the null hypothesis that active application would not affect the dentin bond durability, regardless of etching mode, was rejected. Active application of universal adhesives may enhance dentin bond durability as a result of the potential of tooth demineralization, penetration, and chemical bonding with the dentin substrate.

In self-etch mode, the gel-like collagen in the dentin smear on the sound tissue can interfere with the penetration of resin monomers contained in adhesives.[25,26] SEM observations of treated dentin surfaces in self-etch mode showed that actively applied adhesives can dissolve a certain amount of the smear layer, compared to those applied inactively (Figures 3 and 4). It can be speculated that unreacted H^+ ions were supplied from functional monomers in the adhesive, resulting in the progression of the demineralization process.[4,22] Previous studies[27-29] have reported the benefits of active application for optimal dentin bond performance and durability with self-etch adhesives. Increased dentin bond strength with active application has been suggested[28,29] to be due to the stirring of adhesive-inducing solvent evaporation, resulting in a higher rate of resin monomer incorporation inside the smear layer. Furthermore, the nanolayering of calcium-salt formed from HAp and the functional monomer is significantly greater with active application than with inactive application.[23] The induction of a chemical reaction between functional monomers and HAp by active application may also reduce the levels of the acidic monomer, which would contribute to amine co-initiators and enhance the photopolymerization of adhesives containing acidic functional monomers.[30,31] Therefore, active application in self-etch mode may enhance the dentin bond durability of universal adhesives as a result of the same mechanisms seen with conventional self-etch adhesives.

On the other hand, in etch-and-rinse mode, phosphoric acid pre-etching solubilizes not only the surface debris but also the subsurface of the dentin substrate.[32] A previous investigation[33] of dentin bond durability after four-year water storage showed that a three-step etch-and-rinse system resulted in higher microtensile bond strength than did a two-step etch-and-rinse system, and this tendency did not change over four years. The primary bonding mechanism underlying three-step etch-and-rinse systems is thought to be micromechanical interlocking between demineralized exposed collagen fibrils and resin monomers, leading to hybrid layer and resin tag formation.[32] With phosphoric acid pre-etching performed prior to the application of a self-etch adhesive, concerns remain that demineralized dentin without resin impregnation may persist at the bottom of the hybrid layer, which will act as a weaker region in the vicinity of the resin/dentin interface.[5-7] This unstable region increases the risk of biodegradation and biomechanical influences for not only self-etch adhesives but also for universal adhesives.[34,35]

For all the adhesives subjected to different numbers of TC with active application, SBS values



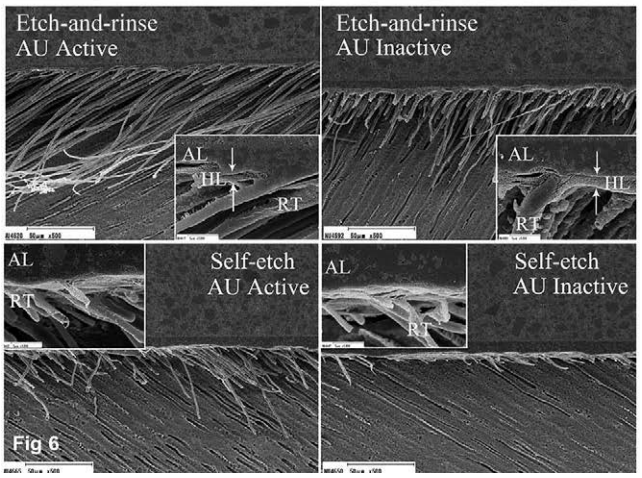


Figure 5. *Representative SEM images of the resin-dentin interfaces. The visible material is indicated by abbreviations: AL, adhesive layer; HL, hybrid layer; RT, resin tag. Upper left image: AB in etch-and-rinse mode with active application method (5000× and 20,000×). Upper right image: AB in etch-and-rinse mode with inactive application method (5000× and 20,000×). Lower left image: AB in self-etch mode with active application method (5000× and 20,000×). Lower right image: AB in self-etch mode with inactive application method (5000× and 20,000×).*

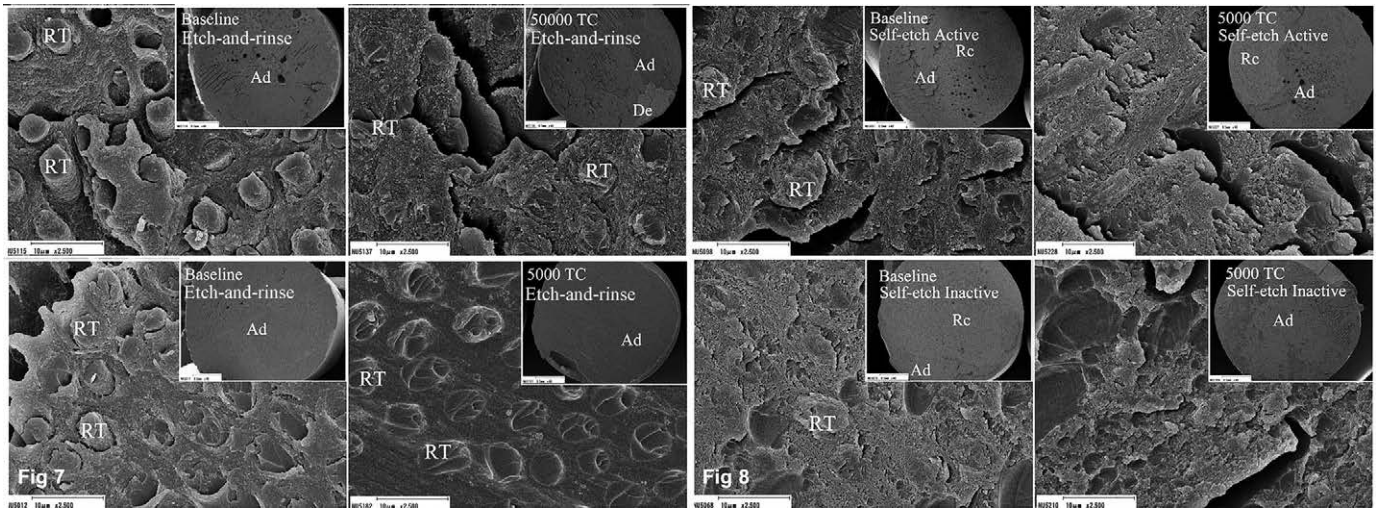
Figure 6. *Representative SEM images of the resin-dentin interfaces. The visible material is indicated by abbreviations: AL, adhesive layer; HL, hybrid layer; RT, resin tag. Horizontal arrows indicate aggregated fillers. Upper left image: AU in etch-and-rinse mode with active application method (5000× and 20,000×). Upper right image: AU in etch-and-rinse mode with inactive application method (5000× and 20,000×). Lower left image: AU in self-etch mode with active application method (5000× and 20,000×). Lower right image: AU in self-etch mode with inactive application method (5000× and 20,000×).*

in etch-and-rinse mode were equal to or greater than those in self-etch mode, with the exception of AU in early cycles. This result was consistent with those of previous studies[2,36] investigating the effects of fatigue stress on the dentin bond durability of universal adhesives in different etching modes. However, SBS values in etch-and-rinse mode with inactive application were lower than those in self-etch mode with active application regardless of the type of adhesive or cycles. Hence, active application is thought to be helpful for the dentin bond durability of universal adhesives in etch-and-rinse mode, similar to the self-etch mode.

The role of resin tag formation in enhancing dentin bond performance is still controversial. SEM images of resin/dentin interfaces in etch-and-rinse mode revealed higher penetration for adhesives with active application (Figure 6). In addition, the penetration of adhesives into the branches of dentin tubules was observed in the active application group, in contrast to behavior in the inactive application group. From the results of the present study, long, thick, and dense resin tags with active application contribute to the higher bond strength due to mechanical retention. Some universal adhesives were designed to have lower hydrophilicity than conventional single-step self-etch adhesives, which may explain the greater resistance of resin tags to hydrolytic degradation. In addition, deep penetration into dentin tubules and creating branches might enhance chemical bonding between functional monomers and the HAp of internal dentin tubules.

The penetration capability of adhesives may be closely dependent on their composition, such as the amount of water, type of solvent, presence or absence of inorganic fillers, and hydrophilic or hydrophobic resin monomers.[37] Although the purity and quantity of each ingredient in the adhesives vary, all the tested adhesives in this study contained the same functional monomer, 10-methacryloyloxydecyl dihydrogen phosphate, the hydrophilic resin monomer 2-hydroxyethyl methacrylate, water, and ethanol as a solvent. However, SEM observations of treated dentin surfaces and resin/dentin interfaces were adhesive dependent. In contrast to AB and SU, greater amounts of deposit were observed on the etched surface in AU (Figure 4).

With regard to resin tag formation, AB showed noticeably longer resin tags than did the other adhesives. This is probably due to its application method, in which the adhesive is applied twice without irradiation between the applications. Thus, the second application may push resin deeper into the substrate. For all the adhesives, no significant SBS reduction was found compared to the baseline until 30,000 TC, regardless of the etching mode or application method used. However, the SBS value for AU in etch-and-rinse mode with inactive application after 50,000 TC was markedly lower than that



Figures 7 and 8. *Representative SEM images of the fractured resin surface in etch-and-rinse and self-etch modes under different storage conditions. The visible material is indicated by abbreviations: Ad, adhesive; De, dentin; Rc, resin composite; RT, resin tag. Figure 7: Upper left image: AU in etch-and-rinse mode with active application method at baseline (40× and 2500×). Upper right image: AU in etch-and-rinse mode with active application method at 50,000 TC (40× and 2500×). Lower left image: AU in total-etch mode with inactive application method at baseline (40× and 2500×). Lower right image: AU in etch-and-rinse mode with inactive application method at 50,000 TC (40× and 2500×). Figure 8: Upper left image: AU in self-etch mode with active application method at baseline (40× and 2500×). Upper right image: AU in self-etch mode with active application method at 50,000 TC (40× and 2500×). Lower left image: AU in self-etch mode with inactive application method at baseline (40× and 2500×). Lower right image: AU in self-etch mode with inactive application method at 50,000 TC (40× and 2500×).*

for all the other conditions. The long tags may explain AB's durability, and SU contains Vitrebond copolymers that utilize glass ionomer technology that may be biocompatible with exposed collagen fibrils.[38] SEM observations revealed that resin monomer penetration in AU was lower than in the other adhesives. This observation is attributed to the presence of aggregated inorganic fillers in AU (Figure 4). Although the incorporation of inorganic fillers enhances the mechanical properties of the adhesive layer, the ability of the adhesive to flow into tubules might decrease.

In terms of clinical practice, this study suggests that active application may enhance the dentin bond durability of universal adhesives regardless of etching mode. However, active application may have a negative impact on immediate enamel bonds in etch-and-rinse mode.[4] Therefore, when using universal adhesives with a combination of different etching modes and application methods, practitioners should take care to select optimal bonding procedures in accordance with the cavity size, depth, and configuration. For instance, when the prepared surface is predominantly enamel, the tested universal adhesives should be applied in etch-and-rinse mode without active application. However, active application should be performed in both etch-and-rinse and self-etch approaches when dentin substrate accounts for a large portion of the prepared surface.

CONCLUSIONS

Within the limitations of this laboratory study, all the factors evaluated, namely application method, adhesive, and thermal cycle period, significantly influenced the SBS values ($p<0.001$), regardless of the etching mode used. For immediate dentin bond results, application method did not strongly influence the dentin bond strengths, regardless of the etching mode. However, for dentin bond durability under thermal cycling stress, application method was a significant influence on dentin bond strengths in both etch-and-rinse and self-etch modes. In conclusion, active application is effective at enhancing the dentin bond durability of universal adhesives.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research Nos. 16K11565, 16K20465, 17K11716, and 17K17141 from the Japan Society for the Promotion of Science. This project was also supported in part by the Sato Fund and by a grant from the Dental Research Center of the Nihon University School of Dentistry, Japan.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee

guidelines and policies of approval of the Nihon University School Dentistry.

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.

(Accepted 12 March 2018)

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Impact of Silane-containing Universal Adhesive on the Biaxial Flexural Strength of a Resin Cement/Glass-ceramic System

F Murillo-Gómez • RB Wanderley • MF De Goes

Clinical Relevance

Using an efficient silane primer is crucial to improve an indirect bonded ceramic restoration's mechanical properties and to preserve interface integrity with resin cement when submitted to load.

SUMMARY

The aim of this study was to determine whether using a silane-containing universal adhesive as a silane primer in glass-ceramic/resin cement systems affects biaxial flexural strength (BFS) and bonded interface integrity after loading. Glass-ceramic (IPS e.max CAD, Ivoclar/Vivadent, Schaan, Liechtenstein) disc-shaped specimens (6.5±0.1mm in diameter, 0.5±0.1mm thick) were etched with 5% hydrofluoric acid (HF) for 20 seconds and divided into four groups of 30 specimens, to be treated as follows: 1) One bottle silane primer (RCP); 2) Separate application of silane and adhesive (RCP+SB); 3) Silane-containing universal adhesive (SBU); 4) No treatment (C). After silanization, all specimens were resin cement– coated and polymerized for 40 seconds. Each specimen layer was measured, as well as each assembly's thickness, using a digital caliper and scanning electron microscope (SEM). Specimens were stored for 24 hours and submitted to a BFS test (1.27 mm/min). BFS values were calculated using the bilayer disc-specimen solution. Bonded interfaces were analyzed on fractured fragments using SEM. One-way ANOVA and Tukey tests (α=0.05) were applied, as well as the Weibull analysis. Factor "silane treatment" was statistically significant ($p<0.0001$). RCP+SB (372.2±29.4 MPa) and RCP (364.2±29.5 MPa) produced significantly higher BFS than did the C (320.7±36.3 MPa) or SBU (338.0±27.1 MPa) groups. No differences were found in the Weibull modulus (*m*: RCP: 10.1-17.3; RCP+SB: 10.1-17.0; SBU: 12.3-22.4; C: 7.4-

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DOI: http://doi.org/10.2341/17-356-L

12.9). Bonded interface analysis exhibited ceramic-cement separation (SBU, C) and voids within the resin cement layer (all groups). Neither the ceramic/cement system's BFS nor its bonded interface stability were improved by SBU after loading.

INTRODUCTION

Metal-free restorations are a suitable clinical option for indirect procedures because of their biocompatibility, excellent mechanical properties, and optimal esthetics.[1] In view of these properties, lithium disilicate glass-ceramic can be considered the material of choice for many clinical situations. To achieve a proper bonding between inorganic restorative materials and organic tooth tissues, coupling agents capable of linking both surfaces must be applied to them.[2-4] Moreover, the use of resin cement to bond ceramic restorations to teeth may improve the mechanical performance at the tooth-ceramic junction.[5,6] To ensure long-lasting tooth-restoration bonding, all these issues must be considered.[7]

Glass-ceramic surface treatment must be performed in order to enhance resin cement/glass-ceramic bonding. Two main approaches are recommended in the literature: mechanical and chemical.[2] The mechanical strategy consists of etching the ceramic surface using hydrofluoric acid (HF), which produces a selective glass-content removal, exposes the crystalline structure, raises surface energy, and facilitates mechanical interlocking of the resin cement.[4,5,8,9] Otherwise, silane couplers provide chemical adhesion between resin cements and silica-containing ceramic substrates.[5] Silane molecules employed in dentistry contain two functional groups: one reacting with polymerizable methacrylates, the other reactive toward silica in glassy structures. The alkoxy groups of this molecule must be activated by a hydrolyzation process (SiOR→SiOH), being suitable then to undergo a condensation reaction when in contact with a ceramic surface, in which water is released as a byproduct.[2] The methacrylate group reacts with the polymerizable side of the resin cement[2] to achieve a three-dimensional, cross-linked network between the ceramic and resin cement.[10-12] This chemical process, complemented with mechanical interlocking, is currently the most accepted procedure for enhancing resin cement/glass-ceramic bonding.[2,4,5]

Following a simpler approach for dental bonding, manufacturers have also added silane to dental adhesives, specifically to a category of materials known as "universal adhesives." These normally contain phosphate acid monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (MDP), which can chemically bond with hydroxyapatite (making it possible to eliminate dentin etching with phosphoric acid), metallic ions present in some ceramics (mainly polycrystalline ceramics), and methacrylate groups of resin cements.[13-15] The addition of silane makes a chemical interaction with glass-containing ceramics possible, amplifying the range of substrates in which these adhesives will act as bonding promoters for resin-based materials.[16] Derived from this, manufacturers claim that silane-containing universal adhesives can be used as conventional silane primers. However, some studies have reported that universal adhesives produced lower ceramic-cement bond strength than conventional silane primers or separate use of silane and adhesive,[17-19] probably because of some kind of silane inactivation inside universal adhesives of low pH or other reason.[19-20]

This broad range of surface treatments, showing different effectiveness, may not only affect glass-ceramic/resin cement adhesion, but its mechanical properties as well. Related to this, the influence of some surface treatment protocols on a material's mechanical performance has also been evaluated. Previous studies have shown an increase in ceramic flexural strength when applying an adhesive, unfilled resin coat,[21-24] or resin cement[12] after a silane coupler. Conversely, another investigation found that the application of conventional silane alone (not resin-cement coated), exerted no effect on ceramic biaxial flexural strength, being more involved with the ceramic's surface texture and unfilled resin application.[25] However, the effect of different types of silane primers (probably showing dissimilar bonding-promoting effectiveness between them) on the flexural strength of ceramic/cement systems is still uncertain.

Thus, the way luting procedures are managed may affect a restoration's bonding and mechanical performance, which are important parameters in understanding clinical behavior.[26] Currently, many options are available to perform glass-ceramic silanization, and manufacturers recommend that they be employed indistinctly (whether silane is mixed with other components or not). To the authors' knowledge, no previous study has evaluated the effect of silane-containing universal adhesives on biaxial flexural strength of a glass-ceramic/resin cement system or the bonding stability they provide to the adhesive interface when submitted to loading forces.

Here we determine whether the use of a silane-containing universal adhesive such as silane primer affects the biaxial flexural strength (BFS) and bonded interface integrity of glass-ceramic/resin cement systems after loading. The null hypotheses tested were that silane-containing universal adhesive does not influence 1) a glass-ceramic/resin cement system's biaxial flexural strength or 2) a glass-ceramic/resin cement system's adhesive interface stability after loading.

METHODS AND MATERIALS

Specimen Fabrication and Group Division

Lithium disilicate CAD/CAM blocks (IPS e.max lithium disilicate CAD/CAM, A2 color, Ivoclar, Vivadent, Schaan, Liechtenstein) were milled on an E4D Dentist System using a cylindrical custom-milled file measuring 6.5 ± 0.1 mm in diameter. Each cylinder was then cut with a diamond saw under water irrigation to obtain discs of 0.5 ± 0.1 mm thickness until completing 120 disc-shaped specimens. Sample measurements were then matched to the appropriate diameter (6.5±0.1 mm) using a digital caliper to fit the biaxial flexure jig. Specimen and flexure jig device dimensions were chosen to simulate the approximate size of a ceramic veneer, as employed in a previous work.[27] Discs were fired unglazed according to the manufacturer's instructions and then ground using #1000 and #2000 grit silicon carbide grinding paper etched with 5% hydrofluoric acid (HF) (Power C Etching, BM4, Palhoça, SC, Brazil) for 20 seconds, water-cleaned for 60 seconds, and ultrasonically cleaned for 5 minutes.

Four groups were formed employing different silanization protocols, treating specimens as follows: 1) RCP (conventional silane): One coat of RelyX Ceramic Primer (3M ESPE, St Paul, MN, USA), a one-bottle conventional silane primer, was actively applied onto the ceramic surface for 60 seconds, followed by thorough drying (20 seconds) using oil-free air until complete solvent evaporation; 2) RCP+SB (conventional silane plus separate adhesive application): one coat of RCP was also applied onto the ceramic surface for 60 seconds and then thoroughly dried (20 seconds) using oil-free air until complete solvent evaporation. Afterward, an adhesive system (Adper Single Bond Plus, 3M ESPE) was applied in one coat for 15 seconds and air-dried for 5 seconds to evaporate the solvent; 3) SBU: Scotchbond Universal (3M ESPE) was applied onto the ceramic surface in one coat for 20 seconds and air-dried for 5 seconds; 4) C: No silane was used and only the previously described HF etching procedure was performed on this group.

After being silanized, all treated surfaces were resin-cement coated (RelyX Ultimate, 3M ESPE). To do so, treated specimens were fixed by the untreated surface on a thick glass plate with the aid of utility wax. One layer of resin cement was placed on the treated surface with the aid of an auto-mixing tip provided by the manufacturer and a micro brush. A polyester strip and a 0.5-mm glass slide were placed on top of the resin cement and pressed using standardized weight devices (200 g) at each side of the glass slide, and at the same time, attached to a digital caliper to control specimen thickness. The resin cement layer was polymerized for 40 seconds (Elipar, S10, 3M ESPE; 800mW/cm^2 as determined using an Ophir Laser measurement potentiometer from Ophir Optronics Ltd., Jerusalem, Israel and taking into account light tip circular area). In the case of groups treated with adhesive systems (RCP+SB and SBU), adhesive and resin cement layers were polymerized simultaneously. Specimen thicknesses were measured again using a digital caliper, and the readings were recorded to calculate each layer's thickness for each specimen (all thicknesses were confirmed using the fractured fragments with the aid of a scanning electron microscope (SEM). Calibration of specimens to fit the flexure jig was performed using a 2000-grit silicon carbide grinding paper whenever necessary. Materials used are described in Table 1.

Biaxial Flexural Strength Test

To measure BFS, the piston-on-ring method was used, employing a customized flexure jig.[27] A schematic representation of the biaxial flexural strength test used in this study is presented in Figure 1. After 24 hour storage in 100% relative humidity at 37°C, all specimens were loosely fitted (resin cement layer facing down) on a support ring (5 mm internal diameter) through a circular aperture (7 mm diameter) of a cylindrical stainless steel jig. Slight specimen flatness imperfections were offset by using a thin piece of rubber film along with a wet piece of filter paper.[28,29] The assembly was positioned on a universal testing machine working at 1.27 mm/min (Instron 4411, Instron Corp, Canton, MA, USA), and a vertical load was applied on the middle of the specimen until failure by a circle-shaped flat piston. The process was monitored and the load recorded at the point of failure, which was used to calculate the BFS/σ_θ according to the bilayer disk approach (considering ceramic and cement as

Table 1: *Materials Used and Their Application Steps*

Material	Type of Material	Manufacturer Lot No.	Composition*	Application Steps
IPS e.max CAD	Lithium disilicate glass-ceramic, A2	Ivoclar, Vivadent, Schaan, Liechtenstein/N76665	SiO_2 , Li_2O, K_2O, P_2O_5, ZrO_2, ZnO, Al_2O_3, MgO, coloring oxides	
RelyX Ceramic Primer (RCP)	Ceramic primer (silane)	3M ESPE St Paul, MN, USA/N406850	MPS, ethanol, water	Apply actively for 60 s, then thoroughly air-dry
Adper Single Bond Plus (SB)	Total-etch adhesive system	3M ESPE Sumaré, SP, Brazil/N334650BR	Bis-GMA, HEMA, dimethacrylates, ethanol,water, photoinitiators, a methacrylate functional copolymer of polyacrylic and polyitaconic acids, and silica nanofiller	Apply actively for 15 s and air-dry for 5 s
Scotchbond Universal (SBU)	Multi-mode adhesive system	3M ESPE St Paul, MN, USA/Neuss, Germany/ 504115	MDP, dimethacrylate resins, HEMA, Vitrebond TM Copolymer, filler, ethanol, water, initiators, silane	Apply actively for 20 s and air-dry for 5 s
RelyX Ultimate	Composite cement, A2	3M ESPE St Paul, MN, USA/Neuss, Germany/ 505370	Base paste: Methacrylate monomers, radiopaque silanated fillers, initiator, stabilizer, rheological additives Catalyst paste: Methacrylate monomers, radiopaque alkaline (basic) fillers, initiator, stabilizer, pigments, rheological additives, fluorescent dye, dark cure activator for Scotchbond Universal	Apply the composite cement with an automixing tip (provided by manufacturer) without separating it from the dispensed mass

** Product composition according to materials safety data sheets (MSDS) provided by the manufacturers.*
MPS, methacryloxypropyltrimethoxysilane (prehydrolyzed silane); MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol A-diglycidyl ether dimethacrylate.

different layers). This method was proposed by Hsueh and others,[29-31] using the analytical model of bilayered disks tested on piston-on-ring device, described by equations 1 to 4[30]:

$$\sigma_\theta = \frac{-PE_2(1+\nu)(z-z_n^*)}{8\pi(1-\nu_2^2)D^*} \times [1 + 2\ln\left(\frac{a}{c}\right) + \frac{1-\nu}{1+\nu}\left(1-\frac{c^2}{2a^2}\right)\frac{a^2}{R^2}] \quad (1)$$

(for $t_1 \leq z \leq t_1 + t_2$ and $r = c$),

where P is the load (N) at fracture, E_2 is the individual Young modulus of layer 2: ceramic (102.7 GPa[32]), z is the axial position of the desired point of calculation on the vertical axis (in this case the axial position used was $z = t_1$ [ceramic/cement interface]), a is the support ring radius (2.5 mm), c is the radius of the indenter of the piston (0.8 mm), and R is the specimen radius (3.25 mm) (Figure 1). The variable ν is given by

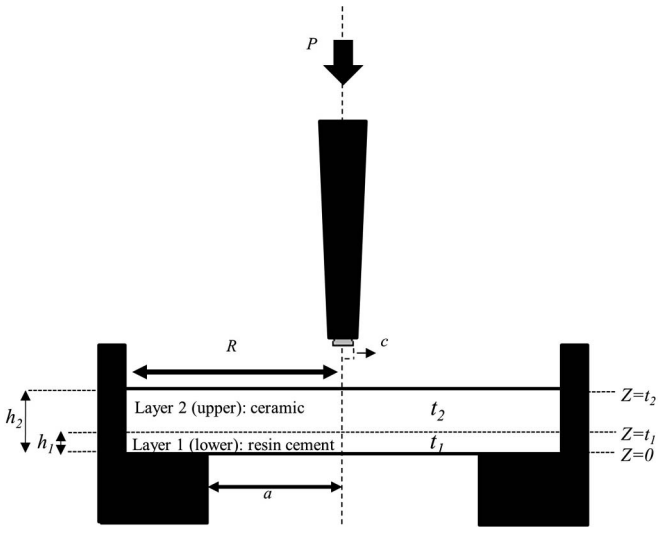

Figure 1. *Schematic representation of the piston-on-ring biaxial flexure test used in this study. Symbology:* P *(load at failure point),* R *(specimen radius),* c *(radius of the indenter of the piston),* t_1 *(individual resin cement layer thickness),* t_2 *(individual ceramic layer thickness),* a *(support ring radius),* z *(axial position).*

Table 2: *Biaxial Flexural Strength Values (BFS) With Standard Deviation (SD) and Weibull Modulus* (m) *With Confidence Intervals (CI) From All Experimental Groups*

Silane Treatment	BFS (SD) (MPa)[a]	*m* (CI)[b]
RCP	364.2 (29.5) A	13.2 (10.1-17.3)
RCP+SB	372.2 (29.4) A	13.1 (10.1-17.0)
SBU	338.0 (27.1) B	16.6 (12.3-22.4)
C	320.7 (36.3) B	9.8 (7.4-12.9)

[a] *Different capital letters represent statistical differences in BFS among the treatments (Tukey,* $p \leq 0.05$*).*
[b] *For* m*, no differences were found. Symbology: Biaxial flexural strength (BFS), standard deviation (SD), confidence interval (CI), RelyX Ceramic Primer (RCP), RelyX Ceramic Primer, and Adper Singlebond Plus (RCP+SB), Scotchbond Universal (SBU), and control (C).*

$$\nu = \frac{\nu_1 t_1 + \nu_2 t_2}{t_{1+} t_2} \tag{2}$$

which is an average considering Poisson ratios from each material (0.215 for ceramic [v_2][specifically for E.max CAD[32]], and 0.27 for resin cement [v_1][33]) and each layers' thickness (t_1: individual resin cement layer thickness; t_2: individual ceramic layer thickness). Each specimen's thickness was measured individually. Variable z^* represents the position of the neutral plane and is given by

$$z_n^* = \frac{\frac{E_1 t_1^2}{2(1-\nu_1^2)} + \frac{E_2 t_2^2}{2(1-\nu_2^2)} + \frac{E_2 t_1 t_2}{1-\nu_2^2}}{\frac{E_1 t_1}{1-\nu_1^2} + \frac{E_2 t_2}{1-\nu_2^2}} \tag{3}$$

where E_1 is the Young's modulus of layer 1: resin cement (10 GPa[28]) and variables t_1, t_2, v_1, v_2 and E_2 are the same as used in equations 4 and 5. D^* is the flexural rigidity, described by

$$D^* = \frac{E_1 t_1^3}{3(1-\nu_1^2)} + \frac{E_2 t_2^3}{3(1-\nu_2^2)} + \frac{E_2 t_1 t_2 (t_1 + t_2)}{1-\nu_2^2} - \frac{\left[\frac{E_1 t_1^2}{2(1-\nu_1^2)} + \frac{E_2 t_2^2}{2(1-\nu_2^2)} + \frac{E_2 t_1 t_2}{1-\nu_2^2}\right]^2}{\frac{E_1 t_1}{1-\nu_1^2} + \frac{E_2 t_2}{1-\nu_2^2}} \tag{4}$$

All fragments were collected, identified by specimen, and the number of fragments obtained from each sample was recorded.

Statistical Analysis

Data normality and homoscedasticity were assessed using the Anderson-Darling and Bartlett tests, both at a preset alpha of 0.05. Results were statistically analyzed using a one-way ANOVA (silane treatment) followed by the Tukey pairwise *post hoc* test, performed at a preset alpha of 0.05. Weibull parameters and distribution plots were also generated (Minitab v18.1, Minitab Inc, State College, PA, USA).

Fractured Fragment Interface Analysis

Fractured fragments were mounted on aluminum stubs, sputter coated with gold/palladium (SCD 050; Balzers, Schaan, Liechtenstein), and then examined using a SEM (JSM 5600 LV; JEOL, Tokyo, Japan), operating at 15 kV and a working distance of 20 mm. Images of representative areas of each fragment were obtained to evaluate interfacial characteristics for each group. In addition, each layer's thickness was measured to confirm initial measurements taken during specimen preparation and recorded using the SEM software.

RESULTS

Statistical analysis showed that the data were normally distributed (Anderson-Darling test [p=0.325]); also, homoscedasticity was proved (Bartlett test [p=0.426]), both at a preset alpha of 0.05, indicating allowable use of parametric methods for data analysis. One-way ANOVA statistical analysis revealed that the factor "silane treatment" significantly influenced BFS (p<0.0001). RCP+SB showed the highest BFS mean, showing no statistical difference from the one obtained by RCP (Table 2). SBU presented a lower BFS mean value than RCP+SB and RCP, but not different from C (Table 2).

No statistical differences were found in m, as all confidence intervals overlapped at least at one point (Table 2). Although not statistically significant, the highest m/graph slope was obtained by SBU and the lowest m/graph slope by the C group (Table 2 and Figure 2).

Representative images from fractured specimen analysis are summarized in Figures 3 to 6. Fractured fragment analysis revealed ceramic-cement separation for groups SBU and C (Figures 5 and 6). In the particular case of SBU (Figure 5), the failure line was located mostly between the adhesive layer and the ceramic material. Conversely, groups RCP and RCP+SB showed a uniform interlocking area (resin cement/ceramic [RCP] and resin cement/adhesive/ceramic [RCP+SB/RC]), in which no gap or interruption of continuity were noted (Figures 3 and 4). Additionally, some voids were noticeable within the resin cement layer of all groups.

DISCUSSION

This study shows that silanization using a silane-containing universal adhesive produced lower BFS

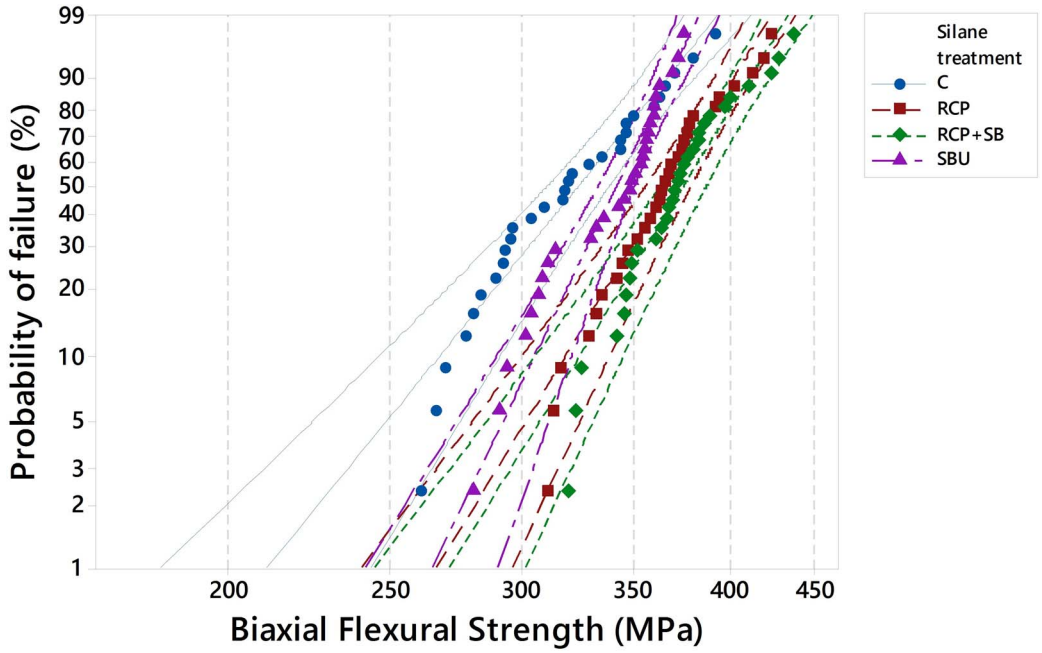

Figure 2. *Weibull distribution corresponding to BFS data. RCP: One-bottle silane primer (RelyX ceramic primer); RCP+SB: Silane and separate application of adhesive (RelyX Ceramic Primer/Adper Single Bond Plus); SBU: Silane-containing universal adhesive (Scotchbond Universal); C: negative control. Lines drawn represent the Weibull curve shape for each group.*

values on ceramic/cement systems than conventional silane and separate application of silane and adhesive. Consequently, the first null hypothesis must be rejected. Because the use of silane-containing universal adhesive as ceramic primer negatively affected the integrity of ceramic/cement adhesive interface during loading, null hypothesis 2 was also rejected. Thus, it can be said that regarding a glass-ceramic/resin cement assembly's mechanical properties, the way resin cement is bonded to the ceramic material may be a very relevant aspect. Additionally, the role of an efficient silane coupling agent appears to be funda-

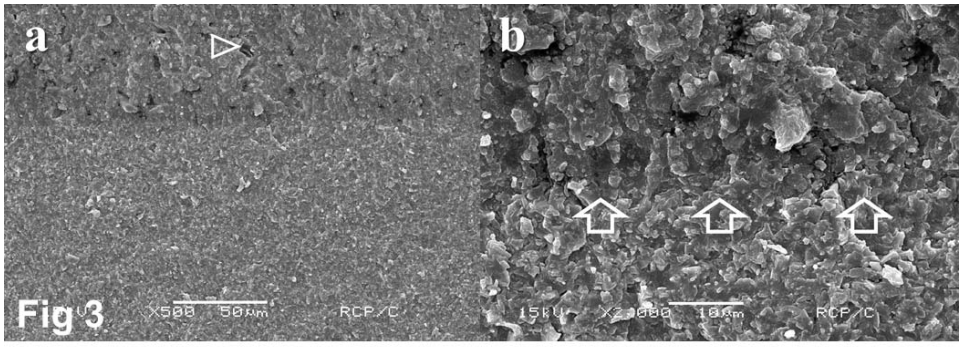

Figure 3. *Representative SEM micrographs of the more prevalent patterns observed in transverse bonded area of the fractured fragment corresponding to group RCP: a) 500 × magnification, showing interlocking of resin cement on ceramic surface and a continuous interface between both materials. Also, some voids within the resin cement layer (triangle pointer), b) close-up from figure 3a (2000×), where ceramic-cement interlocking area is marked between arrows.*

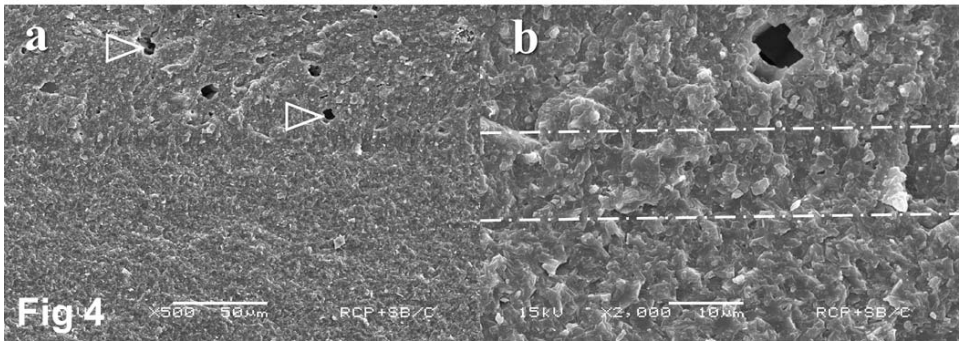

Figure 4. *Representative SEM micrographs of the more prevalent patterns observed in transverse bonded area of the fractured fragment corresponding to group RCP+SB: a) 500× magnification, showing a continuous ceramic-adhesive-cement interlocking and some voids (triangle pointers) within the resin cement layer, b) close-up of Figure 4a (2000 ×), approximate region where ceramic-adhesive and adhesive-cement interlocking areas are located.*

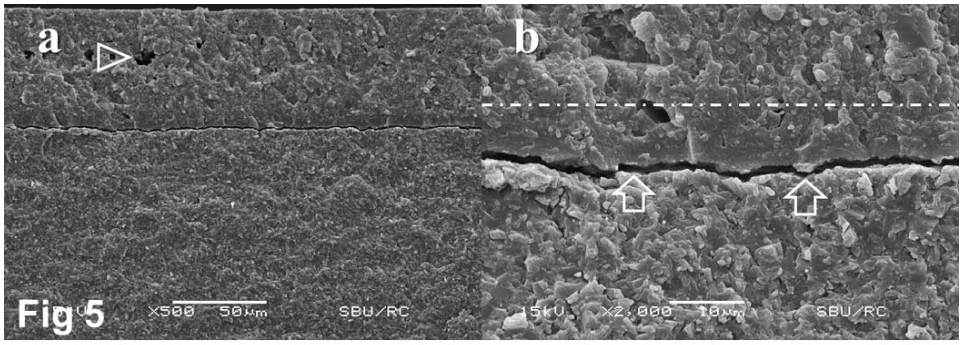

Figure 5. *Representative SEM micrographs of the more prevalent patterns observed in transverse bonded area of the fractured fragment corresponding to group SBU: a) 500 × magnification, showing a separation between the adhesive layer and ceramic material and some voids within resin cement layer (triangle pointer; (500×), b) close-up from Figure 5a (2000×), showing in greater detail the adhesive-cement (pointed line) and ceramic-adhesive interfaces (arrows).*

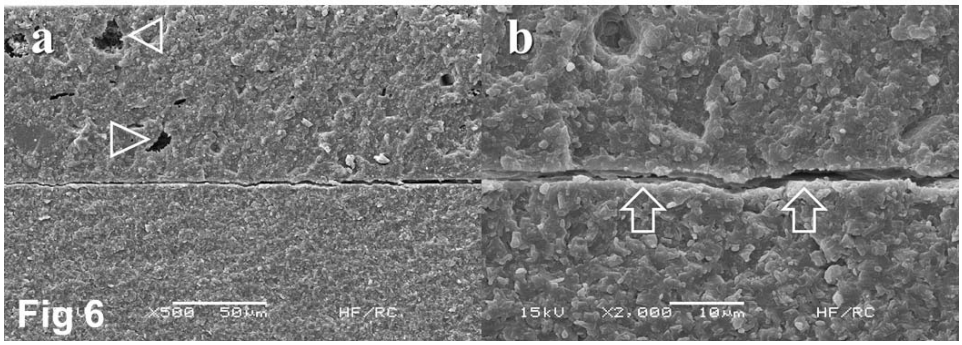

Figure 6. *Representative SEM micrographs of the more prevalent patterns observed in transverse bonded area of the fractured fragment corresponding to group C: a) 500× magnification exhibiting some voids within the resin cement layer (triangle pointers), b) close-up of Figure 6a (2000×), showing a clear separation between the resin cement and the ceramic material (arrows).*

mental in maintaining the integrity of the materials composing the ceramic indirect restoration system.

In order to analyze the mechanical properties of a glass-ceramic/resin cement system influenced by different types of silane primers, the biaxial flexural strength was evaluated using a piston-on-ring biaxial flexure strength test. The biaxial flexure test is more reliable than uniaxial tests, as it applies the stress on a concentric point of the specimen, resulting in a more uniform analysis of material strength.[24,34] The traditional approach for this kind of bending test is not useful to calculate the BFS of multilayered specimens composed of more than one dissimilar material, as it fails to consider the Poisson ratio and individual thickness of each material.[29-31] The analytical solution proposed by Hsueh and others[28-30] includes this possibility, and it is also efficient in calculating biaxial stresses through ceramic/cement bilayered specimens.[28] In the particular case of ceramic specimens treated with an adhesive system layer before resin cement coating (RCP+SB and SBU), although these specimens are composed of three different materials, they were treated as bilayered specimens for calculation purposes, as adhesive layer thickness in this study was recorded to be under 15 μm in all specimens. Such a small thickness would not influence the calculation outcomes.[28] Furthermore, as adhesive and resin cement layers are both composed of resin-based materials and were polymerized together, they were observed to be well integrated; sometimes it was even difficult to differentiate between the two. Thus, being equally valid to apply the bilayer approach for those situations,[24,28] adhesive layers were considered as part of resin cement layers in such cases.

The application of conventional silane (alone or adhesive coated) improved BFS, indicating that silane efficiency in enhancing ceramic/cement bonding plays an important role in a ceramic/cement assembly's mechanical properties. This effect might be explained by the fact that silane bonds chemically with glass-ceramic and resin-based materials, maintaining the integrity of the system. This can be confirmed on SEM images, where a full continuity of the ceramic-cement or ceramic-adhesive-cement interfaces is observed (Figures 3 and 4), suggesting this chemical union was successful. This uniform interlocking area between ceramic and luting materials was previously reported to strengthen glass-ceramics.[21,25] Addison and colleagues suggest that, as an explanation to this phenomenon, when a crack is filled by the resin-based material and the whole system is submitted to load, the Poisson effect is expected to occur, producing a slight contraction at the bottom of the crack (due to its geometry), raising the elastic modulus of the resin-based material in this area and equalizing its behavior to that of the ceramic material.[21,25] This cumulative effect within adjacent cracks may strengthen the resin-based material, maintain ceramic/resin unity, and as a consequence, increase the flexural strength of the whole system.[21,25] On the other hand, applying an

adhesive coat after the silane primer may present better wetting and interpenetrating capabilities than would the resin cement due to the adhesive's lower filler content and viscosity, resulting in a better intimacy with the ceramic material.[23,35] Thus, in light of our results, adhesive coating of previously silanized glass-ceramics has a positive effect on the ceramic/cement system's mechanical properties.

Silane-containing universal adhesives are also recommended by manufacturers to be used as silane primers. In the present study, this kind of material demonstrated no positive effect on the biaxial flexural strength, as it showed no statistical difference compared with the negative control group (Table 2). This may be due to some kind of inefficient bonding property demonstrated by the silane contained in these adhesives, incapable of maintaining the integrity of the ceramic-adhesive-cement assembly, as shown in Figure 5. This lack of unity between "bonded" materials may lead to a faster propagation of microcracks, consequently weakening the whole specimen.

In this study, this pattern was observed in the SBU group and in the negative control group in which silane was not applied (Figures 5 and 6). Previous studies have demonstrated that conventional silane (with or without separate applications of adhesive) performs better than silane-containing universal adhesives as a ceramic-cement bonding promoter.[17-19] Universal adhesives contain many ingredients other than silane, resulting in fewer silane molecules per area in contact with the ceramic surface,[36] in contrast to the silane-only containing primer. Intimate contact between the silane and ceramic surface is crucial, as one silane coat contains three oligomer layers,[37] just the first being capable of forming chemical bonds; the outermost layers may be detrimental.[38] Also, elimination of solvents and other byproducts formed during the silane condensation reaction may be hindered through development of a dense polymer network,[39] needing more time to evaporate solvent in universal adhesives as demonstrated in a previous work.[40] Moreover, a more acidic environment of universal adhesives (SBU, pH 2.7; RCP, pH 4.6)[2] may lead to continuous hydrolyzation and reaction of its silane molecules upon storage, and consequently being inactive to some degree before being used, as proven by Yoshihara and others.[20]

Additionally, the type of silane contained in SBU is not specified; it is possible that the silane compound used in those adhesives is not as effective as the well-known methacryloxypropyltrimethoxysilane. All these issues may explain why universal adhesives failed to improve the ceramic/cement system's BFS and maintain its integrity with resin cement coating. However (though not statistically significant), the SBU attained the highest *m* (16.6) among all groups (Figure 2, Table 2), being very distant from the C group in this regard. This may be explained by the fact that, despite the similar behavior that they (SBU and C) showed regarding BFS and adhesive interface integrity (Table 2, Figures 5 and 6), SBU as an adhesive may have better wettability than the resin cement alone and, further, some positive effect (even though low) would be expected from SBU compared with the control group.

Previous studies have stated that resin cement coatings increase the BFS of ceramic materials,[35,41] while silane priming itself does not.[25] Based on our results, it cannot be said that silane enhances ceramic BFS, but silane's effectiveness does affect adhesive interface behavior of a ceramic/cement assembly during load and consequently its mechanical properties. This scenario can be extrapolated to a clinical situation in which an indirect all-ceramic restoration is luted with resin cement without treating the internal ceramic surface using an efficient silane primer. In that case, less likelihood of restoration success can be expected, as shown here by *m* (Figure 2, Table 2). Thus, we may infer that a positive effect of a resin-cement coating on a ceramic/cement assembly's BFS depends on the performance of a proper silanization process. Resin cement coating per se is no guarantee of improved mechanical properties on ceramic/cement systems, as some voids were found across the resin cement layer in all groups (Figures 3 to 6), even though an auto-mixing tip was used, avoiding manual manipulation. Thus, a "perfect" resin cement layer (with no such defects) may be difficult to reproduce in clinical situations, comprising the mechanical reliability of the restoration.

As shown in this study, glass-ceramic/resin cement chemical bonding seems to be crucial not just for bond strength but for a ceramic cement system's mechanical properties, as the integrity of a bilayered system may be considered a more important factor than the strengthening potential of each layer by itself.

CONCLUSIONS

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

1. The silanization protocol influences the biaxial flexural strength of glass-ceramic/resin cement systems.

2. The application of a silane primer (alone or adhesive coated) to the ceramic surface improves the biaxial flexural strength of glass-ceramic/resin cement assemblies, while the application of a silane-containing universal adhesive does not.

Acknowledgements

The authors thank the Brazilian-CAPES, University of Costa Rica, and Brazilian CNPq (Process No. 310650/2013-5) for partly funding this project. This work was used by F.M-G. in partial fulfillment of the requirements for the PhD degree and as part of his PhD thesis.

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 presented in this article.

(Accepted 8 March 2018)

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Does a Self-etching Ceramic Primer Improve Bonding to Lithium Disilicate Ceramics? Bond Strengths and FESEM Analyses

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Clinical Relevance

Etching with hydrofluoric acid (HF) followed by a silane coupling agent may still be the most reliable surface treatment for lithium disilicate ceramics. Clinicians may need to be aware of HF etchants that result in surface overetching.

SUMMARY

Objective: To compare the effect of hydrofluoric acid (HF) vs self-etching ceramic primer on resin cement microshear bond strength (μSBS) and ultramorphology of lithium disilicate (LD) ceramic.

Methods and Materials: LD (IPS e.max CAD, Ivoclar Vivadent) blocks (14×4×2 mm^3) were polished to 1200 grit and assigned to nine groups (n=5): CON: control, no LD surface treatment; IVO: 5.0% HF (IPS Ceramic Etching Gel, Ivoclar Vivadent); VIT: 5.0% HF (Vita Ceramics Etch, VITA Zahnfabrik); FGM: 5.0% HF (Condac Porcelana, FGM); ULT: 9.0% HF (Porcelain Etch, Ultradent); PRM: 9.6% HF (Premier Porcelain Etch Gel, Premier); BIS: 9.5% HF (Porcelain Etchant, Bisco Inc); DEN: 10.0% HF (Condicionador de Porcelanas, Dentsply Brazil); and MEP: self-etching ceramic primer (Monobond Etch & Prime, Ivoclar Vivadent). For all HF groups and control, an MDP-containing silane solution (MB$^+$, Monobond Plus, Ivoclar Vivadent) was applied on rinsing the HF gel and air drying. Three transparent matrices for each specimen were filled with light-cured resin cement (Variolink Veneer, Ivoclar Vivadent). After storage in water for 48 hours at 37°C, specimens were tested in shear mode to measure μSBS. Mode of failure was analyzed at 50×. Statistical analysis included one-way analysis of variance and the Duncan *post hoc* test (α=0.05). Thirty-six additional LD specimens were assigned to the same experimental groups (n=4) and observed under a field-emission scanning

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DOI: http://doi.org/10.2341/17-355-L

electron microscope (FESEM) at magnifications ranging from 10,000× to 100,000×.

Results: IVO resulted in statistically higher mean μSBS than all the other groups. MEP resulted in statistically lower μSBS than all HF groups. The failure mode for MEP was predominantly adhesive. The most frequent failure mode for the HF groups was mixed. CON resulted in 100% pretesting failures. For FESEM, no retentive pattern was observed for CON specimens. MEP resulted in the least pronounced etching pattern, few areas around crystals exhibited a slight increase in retention pattern compared to the control group. All HF gels created microporosities on the LD surface with distinct etching patterns. VIT and DEN resulted in an LD ultramorphology that suggested overetching.

Conclusions: HF etching followed by a silane solution resulted in higher bond strengths than a self-etching ceramic primer. Some HF gels may cause overetching of the LD intaglio surface.

INTRODUCTION

The creation of microporosities on glass-matrix ceramics using hydrofluoric acid (HF)[1] followed by a silane coupling agent[2] has been the standard procedure for adhesive cementation of porcelain restorations. Bonded glass-matrix ceramic restorations mimic the biomechanical properties, strength, and esthetics of the original tooth[3,4] while performing very well in the clinical setting.[4,5]

Lithium disilicate (LD) glass-matrix ceramic is indicated for full- and partial-coverage bonded ceramic restorations. HF partially dissolves the glass-matrix phase by reacting with silicon dioxide.[6] As a result, HF creates a network of microporosities on the LD surface,[7] forming a microretentive pattern for resin cement interlocking,[8] which leads to higher mean bond strengths compared to nonetched LD.[7-9] Silane coupling agents enhance the resin cement bonding by creating a chemical interaction between the silica in the glass phase of the glass-matrix ceramics and the methacrylate groups of the resin cement.[6,10] 10-Methacryloyloxydecyl dihydrogen phosphate (MDP)– containing silane coupling agents have the potential to interact chemically with LD.[11] The presence of MDP may help maintain the stability of the bonds to LD.[12]

Ivoclar Vivadent, the major manufacturer of LD ceramic, recommends etching the intaglio surface with ≈5% HF gel (IVO, IPS Ceramic Etching Gel, Ivoclar Vivadent, Schaan, Liechtenstein) for 20 seconds. Recently, other ≈5% HF gels have become available, specifically indicated to etch LD. These etchants have specific differences, including gel viscosity, color, packaging type (bottle or syringe), buffering capacity, and the presence of sulfuric acid mixed with HF. However, many of these HF gels have not been tested independently on LD. Therefore, it is relevant to compare their effect on the ultramorphology of LD intaglio surfaces and resulting bond strengths.

A self-etching ceramic primer was introduced in 2015 (MEP, Monobond Etch & Prime, Ivoclar Vivadent). The manufacturer claims that this new primer is capable of etching and priming LD without the need for a separate HF etching step and a separate silane coupling agent. The peer-reviewed literature is scarce regarding the ability of this new all-in-one ceramic primer to promote bonding to LD comparable to that obtained with HF-etched and silane-treated LD. The objective of this study was to compare the effect of HF vs self-etching ceramic primer on the resin cement microshear bond strengths (μSBS) to LD ceramic and respective ultramorphology using field-emission scanning electron microscopy (FESEM). The null hypothesis tested was that the etching protocol would not influence the etching pattern and resin cement μSBS to LD.

METHODS AND MATERIALS

Microshear Bond Strength

Ten IPS e.max CAD blocks LT A2/C14 (Ivoclar Vivadent) were cut into 45 rectangular sections (14×4×2 mm^3) using a slow-speed diamond saw (Model 650, South Bay Tech Inc, San Clemente, CA, USA) under water irrigation. After cleaning ultrasonically with distilled water for 10 minutes, LD specimens were fired following the crystallization program recommended by the manufacturer. The resulting LD specimens were positioned in polyvinyl chloride plastic rings and embedded with epoxy resin (Epo-Thin Resin, Buehler Inc, Lake Buff, IL, USA). The LD surfaces were then ground flat with abrasive silicon carbide paper (360, 600, and 1200 grit) for one minute each under water. An acid-resistant, double-sided adhesive tape (Scotch Permanent Double Sided Tape, 3M, St Paul, MN, USA) was perforated with three 0.8-mm-diameter holes and positioned over the LD surface. Nine experimental groups (n=5) were created (Table 1):

Table 1: *Materials, Manufacturers, Batch Numbers, and Compositions*

Material	Brand Name (Manufacturer), Batch Number	Composition
Lithium disilicate glass ceramic	IPS e.max CAD (Ivoclar Vivadent), S17323	SiO_2, Li_2O, K_2O, P_2O_5, ZrO_2, ZnO, other oxides, coloring oxides
Ceramic etchant	IPS Ceramic Etching Gel (Ivoclar Vivadent), S13497	≤5.0% hydrofluoric acid
Ceramic etchant	Vita Ceramics Etch (VITA Zahnfabrik), 42530	≤5.0% hydrofluoric acid and ≤10% sulfuric acid
Ceramic etchant	Condac Porcelana 5% (FGM Produtos Odontológicos), 100915	5.0% hydrofluoric acid
Porcelain etchant	Porcelain Etch (Ultradent Products, Inc), BBHKX	Buffered 9.0% hydrofluoric acid
Porcelain etchant	Premier Porcelain Etch Gel (Premier Dental Products), PE4343-1	9.6% hydrofluoric acid
Porcelain etchant	Porcelain Etchant (Bisco, Inc), 1500002557	Buffered 9.5% hydrofluoric acid
Porcelain etchant	Condicionador de Porcelanas (Dentsply Brasil), 146312H	10.0% hydrofluoric acid
Silane coupling agent	Monobond Plus (Ivoclar Vivadent), U03528	Ethanol, 3-trimethoxysilylpropyl methacrylate, 10-MDP (MDP), sulfide methacrylate
Self-etching ceramic primer	Monobond Etch & Prime (Ivoclar Vivadent), V09349	Butanol, trimethoxypropyl methaycrylate (silane), ≤10% tetrabutylammonium dihydrogen trifluoride, methacrylated phosphoric acid ester, colorant
Light-cured resin cement	Variolink Veneer (Ivoclar Vivadent), S20664	Paste of dimethacrylates, inorganic fillers, ytterbiumtrifluoride, initiators, stabilizers, pigments

- CON: no LD treatment
- IVO: LD etched with 5.0% HF gel (IPS Ceramic Etching Gel, Ivoclar Vivadent)
- VIT: LD etched with 5.0% HF gel (Vita Ceramics Etch, VITA Zahnfabrik H. Rauter GmbH & Co KG, Bad Säckingen, Germany)
- FGM: LD etched with 5.0% HF gel (Condac Porcelana 5%, FGM Produtos Odontológicos, Joinville, Brazil)
- ULT: LD etched with 9.0% HF gel (Porcelain Etch, Ultradent Products, Inc, South Jordan, UT, USA)
- BIS: LD etched with 9.5% HF gel (Porcelain Etchant, Bisco, Inc, Schaumburg, IL, USA)
- PRM: LD etched with 9.6% HF gel (Premier Porcelain Etch Gel, Premier Dental Products, Plymouth Meeting, PA, USA)
- DEN: LD etched with 10.0% HF gel (Condicionador de Porcelanas, Dentsply Indústria e Comércio Ltda, Petrópolis, Brazil)
- MEP: LD treated with a self-etching ceramic primer (Monobond Etch & Prime, Ivoclar Vivadent)

For the HF groups, a drop of gel was applied directly on the LD surface for 20 seconds. The HF gel was thoroughly rinsed off with water from an air-water syringe for 30 seconds. The LD surface was cleaned ultrasonically in distilled water for 180 seconds and air-dried for 60 seconds. For the HF and control groups, one coat of MB^+ (Monobond Plus, Ivoclar Vivadent) silane coupling agent was applied with a small brush, left on the LD surface for 60 seconds, and then air-dried with a strong jet of water- and oil-free air for 10 seconds. For the MEP group, one drop of the self-etching ceramic primer was scrubbed with a small brush for 20 seconds, left on the LD surface for 40 seconds, and then thoroughly rinsed off with air-water spray and air-dried with a strong jet of water- and oil-free air for approximately 10 seconds.

Subsequently, three polyethylene transparent Tygon tubes (Tygon Medical Tubing Formulations 54-HL, Saint Gobain Performance Plastics, Akron, OH, USA), with an internal diameter of 0.8 mm and a height of 0.5 mm, were positioned over the LD surface in each specimen. A light-cured resin cement (Variolink Veneer, shade Medium Value 0, Ivoclar Vivadent) was carefully packed inside each tube, and a clear Mylar matrix strip was placed over the filled Tygon tube and pressed gently into place. The resin cement was light-cured for 40 seconds using an LED light-curing unit (Bluephase N, Ivoclar Vivadent) with a light energy of 48 J/cm^2.

Specimens were stored in water for 48 hours at 37°C. Each specimen was positioned onto the universal testing machine, and a thin orthodontic wire (0.2-mm diameter) was looped around each resin cement cylinder. The setup was aligned to ensure the accurate orientation of the shear forces.[13] The crosshead speed of a universal testing machine (Instron 4444, Instron Corporation, Canton, MA, USA) was set at 1 mm/min, and the specimens were tested until failure. The μSBS (MPa) was calculated by dividing the load at failure by the surface area (mm^2). After testing, the specimens were examined

under an optical microscope (Leica DM4000 M, Leica Microsystems GmbH, Wetzlar, Germany) in dark-field mode at 50× magnification. The failure mode was classified as cohesive in resin cement (CR, failure exclusively within resin cement), adhesive (A, failure exclusively between the resin cement–LD interface), or mixed (M, failure at the resin cement–LD interface that included any size of cohesive failure of the resin cement over the bonding area).

Statistical Analysis

Statistical analysis was carried out using IBM SPSS 22 (IBM, Armonk, NY, USA) statistical software. The Kolmogorov-Smirnov test showed the sample fit the assumption of normality (p=0.129). The Levene test (p=0.232) demonstrated that the sample variances were not different. Subsequently, a one-way analysis of variance was computed, followed by the Duncan *post hoc* test ($p<0.05$).

FESEM Analysis

Four LD blocks were cut into 36 sections (4×4×1 mm^3) using a slow-speed diamond saw (Model 650, South Bay Tech Inc) under water irrigation. After cleaning ultrasonically with distilled water for 10 minutes, specimens were fired following the crystallization program recommended by the manufacturer.

The specimens were assigned to the same nine groups (n=4), except that silane was not used to prevent masking of the LD surface morphology. Specimens were cleaned ultrasonically with distilled water for 180 seconds, air-dried, and left in a vacuum desiccator for 24 hours. Specimens were mounted on aluminum stubs with adhesive carbon tape (PELCO Carbon Conductive Tape, Ted Pella Inc, Redding, CA, USA) and colloidal quick-drying silver paint (PELCO Colloidal Silver, Ted Pella Inc). Sputter coating was carried out with gold palladium by means of a sputter coater (SCD 500 EVN, Bal-Tec AG, Balzers, Liechtenstein) at 40 mA for 40 seconds. Specimens were observed under a FESEM (JSM-6701F, JEOL, Tokyo, Japan) at an accelerating voltage of 5.0 kV and a working distance of 3.0 to 6.8 mm with at magnifications ranging from 10,000× to 40,000×.

RESULTS

Microshear Bond Strength

Mean μSBS ± SD (MPa) and failure mode are displayed in Table 2. Means with different letters indicate a significant difference ($p<0.05$). All CON specimens and two MEP specimens failed prior to testing and were assigned a value of 0 MPa. Mean μSBS ranged from 0 to 15.0 MPa. IVO resulted in statistically higher mean μSBS (15.0±4.1 MPa) than all the other groups. All other HF groups resulted in similar mean μSBS. However, the HF groups resulted in significantly higher mean μSBS than MEP (3.8±1.9 MPa).

Table 2: *Group, Etchant, Ceramic Primer, Failure Mode (%), and Mean μSBS ± SD. (MPa)*

Group	Etchant	Ceramic Primer	Failure Mode (%)	Mean[a] ± SD
CON	None	Monobond Plus Ivoclar Vivadent	A = 100 M = 00 CR = 0 CC = 0	0.0 ± 0.0 D
IVO	≤5% HF Ivoclar Vivadent		A = 40 M = 47 CR = 13 CC = 0	15.0 ± 4.1 A
VIT	≤5% HF <10% sulfuric acid VITA Zahnfabrik		A = 33 M = 67 CR = 0 CC = 0	8.1 ± 2.7 B
FGM	5% HF FGM Produtos Odontológicos		A = 27 M = 67 CR = 6 CC = 0	7.6 ± 1.7 B
ULT	9% HF Ultradent Products, Inc		A = 34 M = 60 CR = 6 CC = 0	8.0 ± 2.2 B
PRM	9.6% HF Premier Dental Products		A = 14 M = 80 CR = 6 CC = 0	8.5 ± 2.6 B
BIS	9.5% HF Bisco, Inc		A = 27 M = 73 CR = 0 CC = 0	8.6 ± 2.0 B
DEN	10% HF Dentsply Brasil		A = 40 M = 60 CR = 0 CC = 0	8.7 ± 2.8 B
MEP		Monobond Etch & Prime Ivoclar Vivadent	A = 73 M = 27 CR = 0 CC = 0	3.8 ± 1.9 C

[a] *Means with the same letter are not significantly different at* $p < 0.05$.
Abbreviations: A, adhesive; M, mixed; CR, cohesive in resin; CC, cohesive in ceramic.

FESEM Analysis

Figure 1 shows the morphology of untreated LD or CON (Figure 1A), LD treated with HF (IVO [Figure 1B], VIT [Figure 1C], FGM [Figure 1D], ULT [Figure 1E], BIS [Figure 1F], PRM [Figure 1G], DEN [Figure

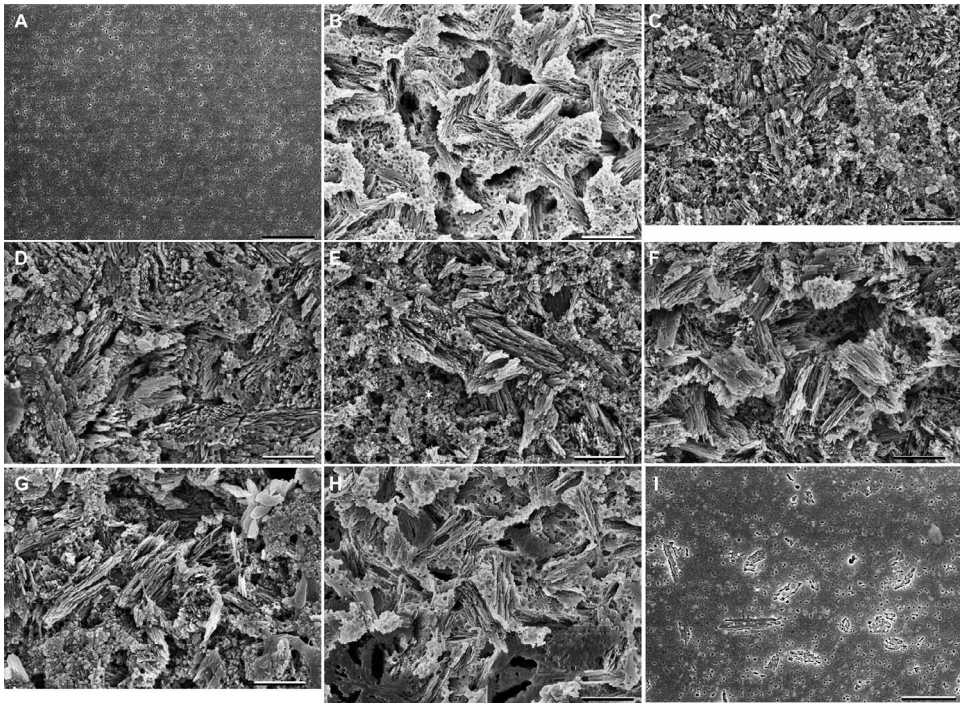

Figure 1. *FESEM micrographs of LD surface after the following treatments: (A): No surface treatment (CON). (B): 5.0% HF (IVO). (C): 5.0% HF (VIT). (D): 5.0% HF (FGM). (E): 9.0% HF (ULT). (F): 9.5% HF (BIS). (G): 9.6% HF (PRM). (H): 10.0% HF (DEN). (I): Self-etching ceramic primer (MEP). Asterisks in Figure 1E denote residual surface deposits. Bar = 1 μm, original magnification = 20,000×.*

1H]), and LD treated with MEP (Figure 1I) at 20,000×. MEP resulted in the least pronounced etching pattern (Figure 1I).

Figure 2 shows a comparison between CON (Figure 2A) and MEP (Figure 2B) at 40,000×. The morphology of LD in the control group showed a smooth surface without retentive features in which 5- to 40-nm-wide nanoporosities were depicted (Figure 2A). In MEP, the LD surface displayed nanoporosities similar to those in the control group but with a larger diameter (20 to 90 nm), while sporadic areas around crystals exhibited a slight increase in retention pattern (Figure 2B) compared to the control group when observed at high magnification (Figure 2A). Comparing the FESEM morphology of the polished LD surfaces (CON) with that of MEP-treated LD surfaces, the ultrasonic cleaning for 180 seconds was able to remove any residual monomer that might have been left by the self-etching ceramic primer MEP (Figure 2B).

Figure 3 shows the morphology of LD treated with VIT (Figure 3A) and FGM (Figure 3B). Figure 3C depicts a high magnification of LD treated with IVO. Figure 4 shows higher magnifications of the morphology of LD treated with BIS (Figure 4A) and with DEN (Figure 4B).

LD specimens treated with IVO (Figure 1B) and FGM (Figure 1D) displayed a more defined etching pattern when compared with ULT (Figure 1E), which displayed the least pronounced etching pattern among the HFs tested, with residual surface deposits resembling a precipitate (Figure 1E). Additionally, IVO resulted in the most consistent and homogeneous etching pattern with an array of

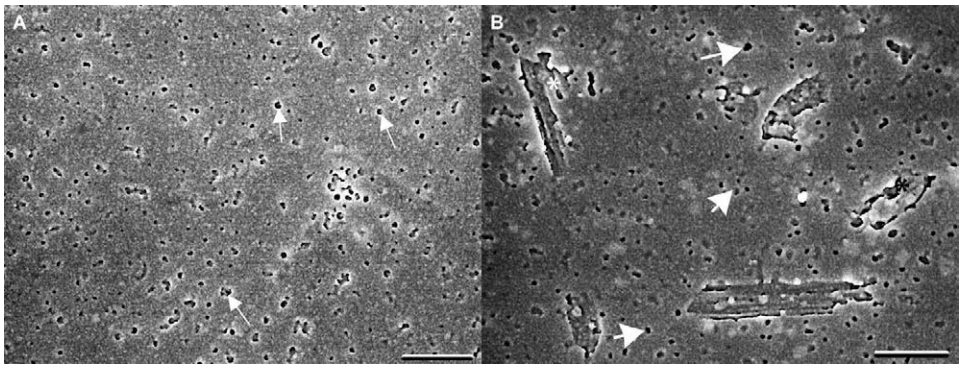

Figure 2. *FESEM micrographs of LD surface (A) without surface treatment (CON) and (B) after treatment with self-etching ceramic primer (MEP). Arrows in Figure 2A show 5- to 40-nm-wide nanoporosities. Arrows in Figure 2B show 20- to 90-nm-wide nanoporosities; asterisks show sporadic retention areas around crystals. Bar = 0.5 μm, original magnification = 40,000×.*

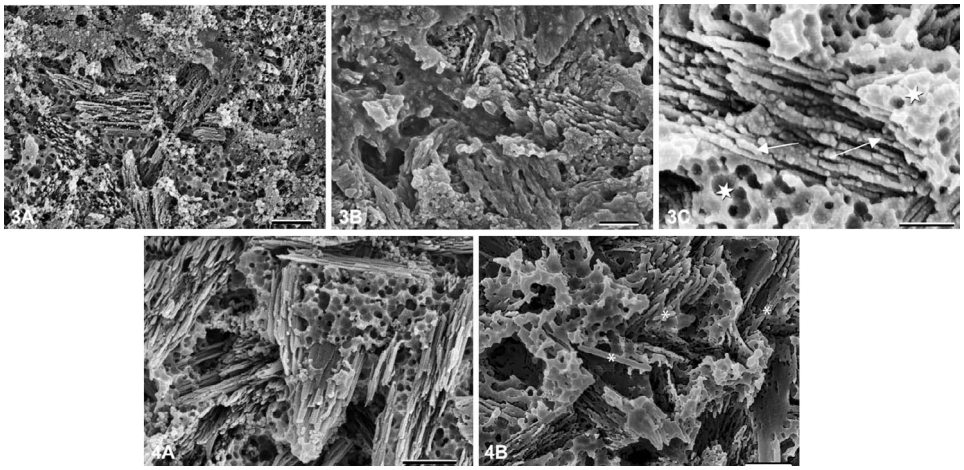

Figure 3. *FESEM micrographs of LD surface after (A) 5% HF (VIT) and (B) 5% HF (FGM). Bar = 0.5 μm, original magnification = 30,000×. (C) Higher magnification of micrograph in Figure 1B (IVO). The glass phase has a honeycomb-like morphology (stars). Arrows show LD crystals attached to the glass phase. Bar = 0.2 μm, original magnification = 100,000×.*

Figure 4. *FESEM micrographs of LD surface after the following treatments: (A): 9.5% HF (BIS). (B): 10.0% HF (DEN). Arrows in Figure 4A and 4B show areas resembling overetching. Pointer in Figure 4B shows unsupported LD crystals. Asterisks in Figure 4B show areas of the glass phase that were detached from the ceramic core, while loose crystals displayed a smoother surface texture. Bar = 0.5 μm, original magnification = 40,000×.*

exposed crystals without apparent overetched areas (Figure 1B). LD crystals were attached to the glass phase, and the latter showed a honeycomb-like morphologic characteristic at 100,000× (Figure 3C). One LD specimen treated with FGM showed areas (Figure 3B) without the same homogeneous etching pattern as seen in Figure 1D. Figure 3B also shows an area where the honeycomb-like glass phase ceramic pattern was not as clear as seen in Figure 1D.

VIT showed areas resembling overetching (Figure 3A). An intermediate deep etching pattern was obtained with BIS (Figures 1F and 4A) and PRM (Figure 1G). DEN specimens displayed the most pronounced etching pattern with unsupported LD crystals and areas with morphology compatible with overetching (Figure 1H). Additionally, areas of the glass phase were detached from the ceramic core, while loose crystals displayed a smoother surface texture at 100,000× (Figure 4B).

DISCUSSION

The null hypothesis was rejected. The "all-in-one" self-etching ceramic primer tested (MEP) resulted in statistically lower mean μSBS to LD than any of the HF etchants. MEP also resulted in the least pronounced etching pattern compared to the groups in which HF was used, which may preclude a durable micromechanical bonding.

MEP contains tetrabutylammonium dihydrogen trifluoride, trimethoxypropyl methaycrylate (silane), and methacrylated phosphoric acid ester.[14] Several sources of fluoride have been investigated for ceramic etching, including acidulated phosphate fluoride, titanium tetrafluoride, and ammonium bifluoride.[15-20] Tetrabutylammonium dihydrogen trifluoride in MEP, also a source of fluoride, is based on ammonium bifluoride (ABF), which is less toxic[21] and less hazardous than HF.[16] It has been reported that the etching patterns of ABF are very similar of those created when HF is applied for a shorter time and at a lower concentration. Therefore, the interaction of ammonium fluoride–based porcelain etchants with ceramics may be similar to that of a low concentration of HF acid applied for a short period.[6] Although ABF was more effective on Dicor castable glass ceramic (DICOR, Dentsply International, York, PA, USA) than HF,[16] etching current glass-matrix ceramics with HF results in statistically higher mean tensile bond strengths compared to etching with ABF.[19] This is in agreement with the results of our study, as MEP contains ammonium fluoride.

Recent studies have reported that mean μSBS were statistically similar when 5% HF was compared with MEP.[22,23] Conflicting results may be explained by differences in testing methodology. For example, the present study used LD polished up to 1200-grit silicon carbide paper for μSBS and FESEM analysis. A pilot study in our laboratory revealed that HF etches glass-matrix ceramic deeper with residual crevices remaining on the surface when specimens are not polished to fine-grit sandpaper. While the LD intaglio surface may be rougher in a clinical situation than in laboratory studies, polished LD is used in the laboratory for standardization purposes.[24-29] Highly polished LD may result in more

consistent ceramic ultramorphology, making it possible to compare the LD etching pattern among different experimental groups. The ultramorphology of LD milled with a standard bur and treated with HF or with MEP was recently evaluated.[30] MEP resulted in a slight increase in micromechanical retention only in the groove areas created during the milling process.[30] As a result of this minor morphological difference, the mean μSBS to milled LD might be slightly higher than that obtained to polished LD. However, the milling process results in a less uniform surface, as it is not possible to standardize the LD surface. This lack of standardization may cause a wider standard deviation of bond strengths for milled surfaces.

In the present study, 10% HF (DEN) specimens displayed the most pronounced etching pattern with unsupported crystals and areas with morphology compatible with overetching. Similarly, VIT showed areas resembling overetching, which may be a result of the presence of <10% sulfuric acid in the composition of VIT.[31] Other studies have reported potential overetching with other HF etchants, especially when used for prolonged etching times. A study using scanning electron microscopy/atomic force microscopy showed that etching with 9.6% HF gel resulted in preferential dissolution of the glass matrix, but the extent of LD surface etching depth increased with etching time.[25] Partially supported crystals within the glass matrix were lost with an etching time longer than 20 seconds. LD became progressively more irregular with numerous voids forming with increasing HF etching time.[25] Another study reported that LD etched with 9.0% HF gel (ULT) for 120 seconds resulted in lower flexural strength than unetched LD,[28] which suggests that overetching LD may weaken the restoration. Xiaoping and others[29] observed that etching LD with 9.5% HF (BIS) for 120 seconds dissolved much of the glass matrix, causing unsupported crystals and an increase in the number of microdefects. Zogheid and others[32] reported that etching LD with 4.9% HF (IVO) for 20 seconds did not result in a significant reduction of flexural strength compared to that of unetched LD. However, etching LD for 90 or 180 seconds with IVO resulted in a significantly lower mean flexural strength compared to that of LD etched for 20 seconds.[32]

Mean μSBS to HF-etched and silane-coated LD using light-cured resin cement obtained in our study are in agreement with other authors.[33,34] Recently, Perdigão and others[33] measured a mean μSBS of 14.7 MPa to HF-etched and MDP-containing silane (MB^+)-coated LD using a light-cured resin cement. Using 10% HF (DEN) to HF-etched and MDP-free silane-coated LD, Baratto and others[34] reported a mean μSBS of 12.5 MPa also using a light-cured resin cement. The light-cured resin cement resulted in lower μSBS than the dual-cure resin cement used in the same study.[34] Lise and others[8] used dual-cure resin cements (Variolink II; Multilink Automix, Ivoclar Vivadent; RelyX Unicem 2, 3M ESPE, St Paul, MN, USA) applied to LD etched with IVO and coated with MB^+. These authors obtained a mean bond strength greater than 40 MPa,[8] most likely as a result of the higher flexural strength and elastic modulus of dual-cure resin cements when light cured.[35] Variolink Veneer (Ivoclar Vivadent) is a microfilled resin cement[33] with a modulus of elasticity of 4.4 ± 0.4 GPa,[36] while RelyX Unicem 2 (3M ESPE) and Variolink II (Ivoclar Vivadent), both dual-cured resin cements, have a mean modulus of elasticity of 10.5 ± 0.1 GPa[37] and 11 ± 0.5 GPa,[36] respectively.

Kalavacharla and others[38] used 5% HF (IVO) for 20 seconds or 9.5% HF (BIS) for 60 seconds on LD surfaces. Mean bond strengths were not significantly different. However, on analysis under the FESEM, LD etched with 5% HF for 20 seconds displayed elongated crystals after partial disintegration of the silica matrix, while LD etched with 9.5% HF for 60 seconds showed a more distinct etching pattern with wider areas of dissolved matrix.[38] These authors concluded that LD etched with 5% HF for 20 seconds is the ideal etching protocol to minimize surface damage to LD. These findings corroborate in part the results in the present study. IVO resulted in higher mean μSBS than the other HF gels tested in spite of apparent similar etching patterns between IVO and FGM. It is unclear whether the etching depth might have played a role in the magnitude of bond strengths.

MB^+ contains MDP, which has potential for chemical interaction with LD substrates.[11] However, the role of MDP in adhesion to LD is not clear. An MDP-containing silane resulted in higher resin cement bond strength to LD than MDP-containing universal adhesives after 150 days in water and 37,500 thermal cycles.[12] Using this same aging process, Elsayed and others[39] showed that bond strengths of resin cement to LD etched with 5% HF followed by the application of MB^+ were more stable compared to those of universal adhesives. However, a silane without MDP (Calibra Silane, Dentsply) followed by a simplified adhesive (Prime & Bond NT, Dentsply) resulted in similar bond strengths to LD

after aging compared to MB^+.[39] In the present study, MB^+ applied to CON resulted in 100% pretesting failures. Oh and Shen[26] also reported a mean bond strength of 0 MPa to both 1200-grit-polished LD and air-abraded LD without silane application. Mean shear bond strengths to 600-grit-polished porcelain blocks was 0 MPa without HF etching and silane application.[40] Even for specimens that were roughened with a diamond bur, Duzyol and others[41] obtained 100% pretesting failures to nonetched LD. The benefit of the presence of MDP in the composition of the silane coupling agent may depend on the depth of exposure of the glass phase by HF etching.

CONCLUSIONS

Within the limitations this study, the following conclusions can be made:

- HF etching followed by a silane/MDP solution may be more reliable for adhesion to LD than a self-etching ceramic primer.
- The absence of an etching pattern that resulted from the application of MEP may preclude micromechanical bonding.
- The ultramorphology of etched LD surfaces depends on the specific HF gel used. While ≈9% HF should be used with caution, 10% HF should be avoided, as it results in overetching. For HF gels with concentration of ≈5%, VIT is the most aggressive.

Acknowledgements

The authors would like to thank Ivoclar Vivadent for the donation of IPS e.max CAD, IPS Ceramic Etching Gel, and Monobond Plus and FGM for donation of Condac Porcelana 5%. Special thanks to Deise R. Consoni for technical assistance with the specimen preparation.

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.

(Accepted 20 February 2018)

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Faculty Positions

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Tooth Sensitivity After Dental Bleaching With a Desensitizer-containing and a Desensitizer-free Bleaching Gel: A Systematic Review and Meta-analysis

M Rezende • FM Coppla • K Chemin • AC Chibinski • AD Loguercio • A Reis

Clinical Relevance: The use of bleaching gel with desensitizer does not reduce the risk and intensity of sensitivity after tooth whitening.

doi: https://doi.org/10.2341/17-253-L

Effect of Argon Plasma Surface Treatment on Bond Strength of Resin Composite Repair

APA Ayres • R Hirata • BM Fronza • BB Lopes • GMB Ambrosano • M Giannini

Clinical Relevance: The use of argon plasma application as a surface treatment did not improve the bond strength of composite repairs. The traditional protocol combining sandblasting, silanization, and the use of a hydrophobic bonding adhesive was the most reliable method.

doi: https://doi.org/10.2341/18-050-L

Amalgam Strength Resistance to Various Contaminants

C Roggenkamp • B Choi • J Chung • R Parhizkar • A Pham • R Robles

Clinical Relevance: This study provides understanding of the potential compromise of amalgam strength after maximum contamination exposure during clinical placement.

doi: https://doi.org/10.2341/18-091-L

Delayed Photoactivation of Dual-cure Composites: Effect on Cuspal Flexure, Depth-of-cure, and Mechanical Properties

KO Hughes • KJ Powell • AE Hill • D Tantbirojn • A Versluis

Clinical Relevance: Delaying two minutes before light-curing after placing dual-cure composites can take advantage of shrinkage stress reduction of the slower self-cure component before the more rapid photoactivation without impacting curing depth or mechanical properties.

doi: https://doi.org/10.2341/18-140-L

©Operative Dentistry, 2019, **44-2,** E58-E74

Tooth Sensitivity After Dental Bleaching With a Desensitizer-containing and a Desensitizer-free Bleaching Gel: A Systematic Review and Meta-analysis

M Rezende • FM Coppla • K Chemin • AC Chibinski • AD Loguercio • A Reis

Clinical Relevance

The use of bleaching gel with desensitizer does not reduce the risk and intensity of sensitivity after tooth whitening.

SUMMARY

Objectives: A systematic review and meta-analysis were performed to evaluate the risk and intensity of tooth sensitivity (TS) after dental bleaching with a desensitizer-containing and a desensitizer-free bleaching gel in adult patients. Color change and risk of gingival sensitivity was also evaluated.

Methods: A comprehensive search was performed MEDLINE via PubMed, Scopus, Web of Science, Latin American and Caribbean Health Sciences Literature database (LILACS), Brazilian Library in Dentistry (BBO), EMBASE and Cochrane Library, and System for Information on Grey Literature in Europe (SIGLE) without restrictions to identify randomized clinical trials. Abstracts from the annual conference of the International Association for Dental Research (1990–2016), unpublished and ongoing trials registries, dissertations, and theses were also searched. The quality of the evidence was rated using the Grading of Rec-

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DOI: http://doi.org/10.2341/17-253-L

ommendations: Assessment, Development and Evaluation (GRADE) approach.

Data: After duplicates were removed, 1352 articles were identified. After title and abstract screening, only 47 studies remained for qualitative evaluation. Most of the studies had unclear risk of bias. No difference between groups were observed for the risk ratio of TS (risk ratio = 0.99; 95% confidence interval [CI] = 0.74–1.33); intensity of TS (standardized difference in means [SMD] = 0.04; 95% CI = 0.79–0.70); color change in shade guide units (SMD – 0.04; 95% CI = 0.50–0.42); color change in ΔE* (SMD = 0.41 (95% CI = 0.07–0.89); and risk ratio of gingival irritation (SMD = 1.05; 95% CI = 0.81–1.36). Except for the risk of TS, graded as moderate quality of evidence, all other outcomes were rated as low and very low quality.

Conclusions: Incorporating desensitizers in the bleaching gel did not reduce the risk of TS, and the quality of this evidence was considered moderate. On the other hand, the intensity of TS, color change, and risk of gingival irritation was similar between groups, but the quality of the evidence for these outcomes was graded as low or very low, thus reducing the level of confidence in these outcomes.

INTRODUCTION

An increased demand for esthetic dentistry has been observed in the dental field over the past 10 years. Some popular magazines suggest that whiter teeth make the smile more attractive and improve people's self-esteem.[1] This explains why a recent study showed that approximately 89% of the participants wished to have their teeth whitened.[2]

Currently, two dentist-supervised techniques are available for dental bleaching: at-home whitening[3-5] and in-office bleaching.[6-8] Although studies show the safety and effectiveness of both techniques.[7,9-11] clinicians still face the undesirable side effect of tooth sensitivity (TS) when performing whitening procedures.

Unfortunately, this side effect is very frequent. Studies have reported that bleaching-induced TS affects 67% to 100% of the participants who received in-office bleaching,[6,7,12-14] and 37% to 90% of the participants who performed at-home bleaching.[3-5,10,15-18]

More precise estimates were recently reported.[19] By evaluating the individual patient data of 11 clinical trials involving bleaching, those authors reported that the risk of TS for in-office bleaching when using 35% hydrogen peroxide (HP) (62.9%; 95% confidence interval [CI] 56.0–67.3) and for at-home bleaching with 10% to 16% carbamide peroxide (CP) (51%; 95% CI 41.4.–60.6)[19] were quite similar. On the other hand, the intensity of TS was very different between the bleaching protocols. On a 0 (no pain) to 4 (very intense pain) pain scale, the overall mean intensity of bleaching-induced TS for in-office bleaching was 2.8 ± 2.9, whereas at-home bleaching patients reported sensitivity of 0.5 ± 0.9.[19] This makes the at-home procedure the most recommended bleaching protocol.[20-22]

The presence of TS has led some researchers to evaluate techniques for minimizing or even eliminating bleaching-induced TS. Recently evaluated techniques include reducing the concentration and usage time of the bleaching product,[9,23-25] applying topical desensitizing agents while bleaching,[25-27] and administering systemic medicines.[12,28,29]

Topical application of desensitizing agents appears to be an effective strategy for reducing TS for both at-home bleaching[16,26,30] and in-office bleaching.[31-34] To reduce the application time, some manufacturers have incorporated some desensitizing agents, such as potassium nitrate and sodium fluoride, into the formulation of bleaching gels[35-37] so that the extra step of applying a desensitizing gel could be eliminated.

Few studies have addressed the effectiveness of desensitizer-containing bleaching gels in reducing bleaching-induced TS, while the available studies report conflicting results. Some authors reported a reduction of bleaching-induced TS when desensitizer-containing gels were used,[5,7,9,14,38,39] whereas others reported no significant difference between desensitizer-containing and desensitizer-free bleaching gels.[22,35]

In light of this, the aim of this systematic review of the literature was to answer the following PICO (problem/patient/population, intervention/indicator, comparison, and outcome) question: Are the risk and intensity of TS in adult patients who have submitted to dental bleaching lower when desensitizer-containing products are compared with desensitizer-free bleaching products?

METHODS AND MATERIALS

Protocol and Registration

This study was registered at the PROSPERO under protocol number CRD 42016036411. For this report,

we followed the recommendations of the PRISMA statement for systematic reviews.[40]

Information Sources and Search Strategy

The controlled vocabulary (MeSH terms) and free keywords in the search strategy were defined based on the PICO question described earlier. For the primary outcomes, we evaluated the risk and intensity of TS during dental bleaching as measured using the visual analog scale (VAS) and numerical rating scale (NRS), during and up to 24 hours after dental bleaching (ie. the period during which patients are more prone to experiencing TS). Color change in the shade guide units (SGUs) and in ΔE (CIEL*a*b* system), as well as the risk of gingival sensitivity, were also evaluated as secondary outcomes.

The following electronic databases were used to identify eligible studies: Cochrane Library, MEDLINE via PubMed, EMBASE, Latin American and Caribbean Health Sciences Literature database (LILACS), and Brazilian Library in Dentistry (BBO). The authors also searched the following citation databases: Scopus and Web of Science (Table 1). The reference lists of all primary studies were searched for additional relevant publications and the related articles link of each primary study in the PubMed database. No restrictions on publication date or languages were imposed.

Grey literature, which is not available through the usual bibliographic sources like databases or indexes, was also investigated (eg, unpublished observations, dissertations, conference proceedings)[41-43] The abstracts of the annual conference of the International Association for Dental Research and its regional divisions (1990–2016) were explored. The System for Information on Grey Literature in Europe (SIGLE), ProQuest Dissertations and Theses full-text database, and Periodicos CAPES theses were also included in the search strategy.

To locate unpublished and ongoing trials related to the review question, the following clinical trial registries were searched: Current Controlled Trials, International Clinical Trials Registry Platform, ClinicalTrials.gov, Registro Brasileiro de Ensaios Clinicos (ReBec), and EU Clinical Trials Register.

Eligibility Criteria

Parallel and split-mouth randomized clinical trials that compared the risk and intensity of TS during in-office and at-home dental bleaching in adult patients of any age group were included. Randomized clinical trials were excluded if: 1) the studies evaluated the effect of topical desensitizing agents containing potassium nitrate and/or sodium fluoride; 2) the studies did not have both groups under investigation; or 3) the studies included both groups but did not compare bleaching gels with equivalent concentrations.

Initially, the articles were selected based on their titles and abstracts according to the previously described search strategy, with duplicates removed. Full-text articles were also obtained, and subsequently, three reviewers (MR, FMC, and KC) classified those that met the inclusion criteria. To handle many studies, a study identification was given to each eligible study, combining the first author and the year of publication.

Data were extracted using customized extraction forms that included details about study methods, designs and settings, age and sex of the participants, details regarding the bleaching protocol, bleaching agents, and type of desensitizing gel added in the included studies.

If there were multiple reports of the same study (ie, reports with different follow-ups), data from all reports were extracted directly into a single data collection form to avoid overlapping data. When data were not reported in the studies, the authors were contacted by email at least twice to request the missing information.

When data from multiple bleaching sessions were provided, an average of the figures was calculated for each bleaching protocol. When evaluating color change, the data that represented the most immediate result (up to 3 months' after bleaching) was used. When more than one bleaching agent from the same group was included in the study, their values were combined to make a single entry.

Risk of Bias in Individual Studies

Three independent reviewers (MR, FMC, and KC) performed quality assessments using the Cochrane Collaboration's tool for assessing risk of bias in randomized trials.[44] The assessment criteria contained six domains: sequence generation, allocation concealment, the blinding of the outcome assessors, incomplete outcome data, selective outcome reporting, and other possible sources of bias.

For each aspect of the quality assessment, the risk of bias was scored following the recommendations of the *Cochrane Handbook for Systematic Reviews of Interventions 5.1.0* (http://handbook.cochrane.org). At the study level, studies were at a low risk of

bias if there was adequate sequence generation, allocation concealment, and patient blinding (key domains). The study was considered unclear if any of the aforementioned key domains was unclear. If at least one of the aforementioned domains was at a high risk of bias, the study was considered to have a high risk of bias. When the study was judged as unclear in its key domains, the authors were contacted to obtain more information and to allow a definitive "yes" or "no" judgment.

During data selection and quality assessment, any disagreements among the reviewers were solved through discussion and, if needed, by consulting a fourth reviewer (AR).

Summary Measures and Synthesis of the Results

Data were analyzed using Revman 5 (Review Manager Version 5, The Cochrane Collaboration, Copenhagen, Denmark). Data from eligible studies were either dichotomous (absolute risk of TS and gingival irritation) or continuous (intensity of TS, ΔSGUs, and ΔE). The outcomes were summarized by calculating the standardized mean difference for the continuous data and the risk ratio with a 95% confidence interval (CI).

Random-effects models were employed. Heterogeneity was assessed using the Cochran Q test and I^2 statistics. All analyses were conducted using Revman 5 (Review Manager Version 5, The Cochrane Collaboration, Copenhagen, Denmark). No subgroup analysis was performed. Sensitivity analyses were also conducted to investigate the reasons for high heterogeneity whenever detected.

Assessment of the Quality of Evidence Using GRADE

We graded the quality of the evidence for each outcome across studies (body of evidence) using the Grading of Recommendations: Assessment, Development and Evaluation (GRADE) (http://www.gradeworkinggroup.org/) to determine the overall strength of the evidence for each meta-analysis.[45] The GRADE approach is used to contextualize or justify intervention recommendations with four levels of evidence quality, ranging from high to very low.

The GRADE approach begins with the study design (randomized clinical trials or observational studies) and then addresses five reasons for possibly decreasing the rating of the quality of the evidence by one or two levels (risk of bias, imprecision, inconsistency, indirectness of evidence, and publication bias) and three reasons for possibly increasing the rating of the quality (large effect, management of confounding factors, dose-response gradient).[45] Each one of these topics was assessed as no limitations, serious limitations, and very serious limitations to allow for the categorization of the quality of the evidence for each outcome as: high, moderate, low, and very low. The high quality rank suggests very high confidence that the true effect lies close to the estimate of the effect. On the other extreme, "very low quality" suggests that there is very little confidence in the effect estimate and that the estimate reported can be substantially different from what was measured.

The GRADEpro Guideline Development Tool, available online (www.gradepro.org), was used to create a summary of findings table, as suggested in the *Cochrane Handbook for Systematic Reviews of Interventions*.[41]

RESULTS

Study Selection

After the database screening and the removal of duplicates, 1352 studies were identified (Figure 1). After title screening, 142 studies remained, which were reduced to 47 after a careful examination of the abstracts. Eight studies remained after reading the full text, with the others excluded for the following reasons:

1) Studies without a control[7,17,46-52];
2) Inadequate control group[37,53-56];
3) Studies that used desensitizer-containing and desensitizer-free bleaching gels without equivalent HP/CP concentrations[5,10,22,57-62];
4) Studies that did not have a desensitizer-containing bleaching agent[8,63-69];
5) Studies that involved application of topical desensitizers instead of desensitizer-containing bleaching gels.[70-77]

Characteristics of Included Articles

The characteristics of the eight selected studies are listed in Table 2. The parallel study design was used in five studies[14,35,36,38,78] and the split-mouth design in three studies.[22,39,79]

Four of the eight studies used only a VAS for pain evaluation,[22,36,39,78] two studies used only the NRS pain scale,[14,79] and one study used both the VAS and NRS scales.[38] The study of Browning and others[35] did not evaluate pain intensity.

Table 1: *Electronic Database and Search Strategy*

Pubmed (31/May/2016)		
#1 (Tooth discoloration[MeSH Terms]) OR Dentition, Permanent[MeSH Terms]) OR "tooth staining"[Title/Abstract]) OR "tooth stain"[Title/Abstract]) OR "stained tooth"[Title/Abstract]) OR "stained teeth"[Title/Abstract]) OR "tooth discoloration"[Title/Abstract]) OR "tooth discolouration"[Title/Abstract]) OR "discolored tooth"[Title/Abstract]) OR "discoloured tooth"[Title/Abstract]) OR "discolored teeth"[Title/Abstract]) OR "discoloured teeth"[Title/Abstract]) OR "teeth discoloration"[Title/Abstract]) OR "teeth discolouration"[Title/Abstract]) OR "dental discoloration"[Title/Abstract]) OR "dental discolouration"[Title/Abstract]) OR "tooth discolorations"[Title/Abstract] OR "tooth color"[Title/Abstract] OR "tooth colour"[Title/Abstract] OR "teeth color"[Title/Abstract] OR "teeth colour"[Title/Abstract]	**#2** (Tooth Bleaching[MeSH Terms]) OR Tooth Bleaching agents[MeSH Terms]) OR Peroxides[MeSH Terms]) OR Carbamide peroxide[Supplementary Concept]) OR Hydrogen peroxide[MeSH Terms]) OR dentin desensitizing agents[MeSH Terms]) OR dentin sensitivity[MeSH Terms]) OR Potassium nitrate[MeSH Terms]) OR Glutaral[MeSH Terms]) OR Sodium Fluoride[MeSH Terms]) OR Gluma desensitizer[Supplementary Concept]) OR bleaching[Title/Abstract]) OR whitening[Title/Abstract]) OR "tooth sensitivity"[Title/Abstract]) OR "potassium oxalate"[Title/Abstract]) OR "GLUMA Desensitizer"[Title/Abstract]) OR desensitization[Title/Abstract]) OR glutaraldehyde[Title/Abstract]) OR "potassium nitrate"[Title/Abstract]) OR "dentin sensitivity"[Title/Abstract]) OR "hydrogen peroxide"[Title/Abstract]) OR "carbamide peroxide"[Title/Abstract]) OR "sodium fluoride"[Title/Abstract]) OR "calcium phosphates"[Title/Abstract]) OR "calcium phosphate"[Title/Abstract]) OR "desensitizing agents"[Title/Abstract]) OR "CPP-ACP"[Title/Abstract]	**#3** (randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized controlled trials[mh] OR random allocation[mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR (placebos[mh] OR placebo*[tw] OR random*[tw] OR research design[mh:noexp] OR comparative study[pt] OR evaluation studies as topic[mh] OR follow-up studies[mh] OR prospective studies[mh] OR control*[tw] OR prospective*[tw] OR volunteer*[tw]) NOT (animals[mh] NOT humans[mh]))
#1 AND #2 AND #3		
Scopus (31/May/2016)		
#1 TITLE-ABS-KEY ("tooth stain*") OR TITLE-ABS-KEY ("stained t??th") OR TITLE-ABS-KEY ("tooth discoloration*") OR TITLE-ABS-KEY ("tooth discolouration") OR TITLE-ABS-KEY ("discolored t??th") OR TITLE-ABS-KEY ("discoloured t??th") OR TITLE-ABS-KEY ("teeth discoloration") OR TITLE-ABS-KEY ("teeth discolouration") OR TITLE-ABS-KEY ("dental discoloration") OR TITLE-ABS-KEY ("t??th color") OR TITLE-ABS-KEY ("t??th colour") OR TITLE-ABS-KEY ("dental discolouration")	**#2** TITLE-ABS-KEY (bleaching) OR TITLE-ABS-KEY (whitening) OR TITLE-ABS-KEY ("tooth sensitivity") OR TITLE-ABS-KEY ("potassium oxalate") OR TITLE-ABS-KEY ("GLUMA Desensitizer") OR TITLE-ABS-KEY (desensitization) OR TITLE-ABS-KEY (glutaraldehyde) OR TITLE-ABS-KEY ("potassium nitrate") OR TITLE-ABS-KEY ("dentin sensitivity") OR TITLE-ABS-KEY ("hydrogen peroxide") OR TITLE-ABS-KEY ("carbamide peroxide") OR TITLE-ABS-KEY ("sodium fluoride") OR TITLE-ABS-KEY ("calcium phosphate*") OR TITLE-ABS-KEY ("desensitizing agents") OR TITLE-ABS-KEY ("CPP-ACP")	**#3** (LIMIT-TO (SUBJAREA , "DENT"))
#1 AND #2 AND #3		
Web of Science (31/May/2016)		
#1 TOPIC: ("tooth stain$") OR TOPIC: ("stained t??th") OR TOPIC: ("tooth discoloration$") OR TOPIC: ("tooth discolouration") OR TOPIC: ("discolored t??th") OR TOPIC: ("discoloured t??th") OR TOPIC: ("teeth discoloration") OR TOPIC: ("teeth discolouration") OR TOPIC: ("dental discoloration") OR TOPIC: ("dental discolouration") OR TOPIC: ("t??th color") OR TOPIC: ("t??th colour")	**#2** TOPIC: (bleaching) OR TOPIC: (whitening) OR TOPIC: ("tooth sensitivity") OR TOPIC: ("potassium oxalate") OR TOPIC: ("GLUMA Desensitizer") OR TOPIC: (desensitization) OR TOPIC: (glutaraldehyde) OR TOPIC: ("potassium nitrate") OR TOPIC: ("dentin sensitivity") OR TOPIC: ("hydrogen peroxide") OR TOPIC: ("carbamide peroxide") OR TOPIC: ("sodium fluoride") OR TOPIC: ("calcium phosphate$") OR TOPIC: ("desensitizing agents") OR TOPIC: ("CPP-ACP")	

Table 1: *Electronic Database and Search Strategy (cont.)*

#1 AND #2		
Lilacs and BBO (31/May/2016)		
#1 tw:((mh:(Tooth discoloration)) OR (mh:(Dentition, Permanent)) OR (tw:("tooth color")) OR (tw:("tooth colour")) OR (tw:("teeth color")) OR (tw:("teeth colour")) OR (tw:("tooth staining")) OR (tw:("tooth stain")) OR (tw:("stained tooth")) OR (tw:("stained teeth")) OR (tw:("tooth discoloration")) OR (tw:("tooth discolouration")) OR (tw:("discolored tooth")) OR (tw:("discoloured tooth")) OR (tw:("discolored teeth")) OR (tw:("discoloured teeth")) OR (tw:("teeth discoloration")) OR (tw:("teeth discolouration")) OR (tw:("dental discoloration")) OR (tw:("dental discolouration")) OR (tw:("tooth discolorations")) OR (tw:("color del diente")) OR (tw:("Color de los dientes")) OR (tw:("Manchas en los dientes")) OR (tw:("Manchas dentales")) OR (tw:("Diente manchado")) OR (tw:("dientes manchados")) OR (tw:("Descoloración de los dientes")) OR (tw:("Dientes descoloridos")) OR (tw:("Dientes de descoloración")) OR (tw:("Descoloración dental")) OR (tw:("descoloraciones de los dientes")) OR (tw:("cor dental")) OR (tw:("Cor dos dentes")) OR (tw:("Dente escurecido")) OR (tw:("Dentes escurecidos")) OR (tw:("Dente manchado")) OR (tw:("Dentes manchados")) OR (tw:("Descoloração dos dentes")) OR (tw:("Descoloração dentária")) OR (tw:("Dente descolorido")) OR (tw:("Descoloração dental")) OR (tw:("descoloraçõ̃es dos Dentes")	**#2** mh:(Tooth Bleaching)) OR (mh:(Tooth Bleaching agents)) OR (mh:(Peroxides)) OR (mh:(Hydrogen peroxide)) OR (mh:(dentin desensitizing agents)) OR (mh:(dentin sensitivity)) OR (mh:(Glutaral)) OR (mh:(Sodium Fluoride)) OR (tw:(bleaching)) OR (tw:(Clareamiento)) OR (tw:(blanqueamiento)) OR (tw:("Clareamento dental")) OR (tw:(whitening)) OR (tw:("tooth sensitivity")) OR (tw:("Sensibilidad dental")) OR (tw:("Sensibilidade dental")) OR (tw:("potassium oxalate")) OR (tw:("Oxalato de potasio")) OR (tw:("Oxalato de potássio")) OR (tw:("GLUMA Desensitizer")) OR (tw:("Desensibilizante GLUMA")) OR (tw:("Dessensibilizante GLUMA")) OR (tw:(desensitization)) OR (tw:(desensibilización)) OR (tw:(dessensibilização)) OR (tw:(glutaraldehyde)) OR (tw:(glutaraldehído)) OR (tw:(glutaraldeído)) OR (tw:("potassium nitrate")) OR (tw:("nitrato de potasio")) OR (tw:("Nitrato de potássio")) OR (tw:("dentin sensitivity")) OR (tw:("sensibilidad dentinaria")) OR (tw:("Sensibilidade da dentina")) OR (tw:("hydrogen peroxide")) OR (tw:("peróxido de hidrógeno")) OR (tw:("peróxido de hidrogênio")) OR (tw:("carbamide peroxide")) OR (tw:("Peróxido de carbamida")) OR (tw:("sodium fluoride")) OR (tw:("fluoruro de sodio")) OR (tw:("fluoreto de sódio")) OR (tw:("calcium phosphates")) OR (tw:("fosfatos de calcio")) OR (tw:("fosfatos de cálcio")) OR (tw:("calcium phosphate")) OR (tw:("Fosfato de calcio")) OR (tw:("fosfato de cálcio")) OR (tw:("desensitizing agents")) OR (tw:("agentes desensibilizantes")) OR (tw:("Agente desensibilizante")) OR (tw:("CPP-ACP")	**#3** db: ("LILACS" OR "BBO")
#1 AND #2		
Cochrane Library (31/May/2016)		
#1"tooth staining":ti,ab,kw or "tooth stain":ti,ab,kw or "stained tooth":ti,ab,kw or "stained teeth":ti,ab,kw or "tooth discoloration":ti,ab,kw (Word variations have been searched) #2"tooth discolouration":ti,ab,kw or "discolored tooth":ti,ab,kw or "discoloured tooth":ti,ab,kw or "discolored teeth":ti,ab,kw or "discoloured teeth":ti,ab,kw (Word variations have been searched) #3"teeth discoloration":ti,ab,kw or "teeth discolouration":ti,ab,kw or "dental discoloration":ti,ab,kw or "dental discolouration":ti,ab,kw or "tooth discolorations":ti,ab,kw (Word variations have been searched) #4"tooth color":ti,ab,kw or "tooth colour":ti,ab,kw or "teeth color":ti,ab,kw or "teeth color":ti,ab,kw (Word variations have been searched)	#5Dentition, Permanent #6Tooth discoloration #7#1 or #2 or #3 or #4 or #5 or #6 #9Tooth Bleaching agents #10Peroxides #11Hydrogen peroxide #12dentin desensitizing agents #13dentin sensitivity #14nitrates #15Glutaral #16Sodium Fluoride	#17bleaching:ti,ab,kw or whitening:ti,ab,kw or "tooth sensitivity":ti,ab,kw or "potassium oxalate":ti,ab,kw or "GLUMA Desensitizer":ti,ab,kw (Word variations have been searched)#18desensitization:ti,ab,kw or glutaraldehyde:ti,ab,kw or "potassium nitrate":ti,ab,kw or "dentin sensitivity":ti,ab,kw or "hydrogen peroxide":ti,ab,kw (Word variations have been searched) #19"carbamide peroxide":ti,ab,kw or "sodium fluoride":ti,ab,kw or "calcium phosphates":ti,ab,kw or "calcium phosphate":ti,ab,kw or "desensitizing agents":ti,ab,kw (Word variations have been searched) #20"CPP-ACP":ti,ab,kw (Word variations have been searched) #21#8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 #22#7 and #21
Embase (31/May/2016)		
#1 'tooth discoloration'/exp OR 'secondary dentition'/exp OR 'tooth color':ab,ti OR 'tooth colour':ab,ti OR 'teeth color':ab,ti OR 'teeth colour':ab,ti OR 'tooth staining':ab,ti OR 'tooth stain':ab,ti OR 'stained tooth':ab,ti OR 'stained teeth':ab,ti OR 'tooth discoloration':ab,ti OR 'tooth discolouration':ab,ti OR 'discolored tooth':ab,ti OR 'discoloured tooth':ab,ti OR 'discolored teeth':ab,ti OR 'discoloured teeth':ab,ti OR 'teeth discoloration':ab,ti OR 'teeth discolouration':ab,ti OR 'dental discoloration':ab,ti OR 'dental discolouration':ab,ti OR 'tooth discolorations':ab,ti AND [embase]/lim	**#2** 'tooth bleaching agent'/exp OR 'peroxide'/exp OR 'urea'/exp OR 'hydrogen peroxide'/exp OR 'desensitizing agent'/exp OR 'dentin sensitivity'/exp OR 'potassium nitrate'/exp OR 'glutaraldehyde'/exp OR 'sodium fluoride'/exp OR bleaching:ab,ti OR whitening:ab,ti OR 'tooth sensitivity':ab,ti OR 'potassium oxalate':ab,ti OR 'gluma desensitizer':ab,ti OR desensitization:ab,ti OR glutaraldehyde:ab,ti OR 'potassium nitrate':ab,ti OR 'dentin sensitivity':ab,ti OR 'hydrogen peroxide':ab,ti OR 'carbamide peroxide':ab,ti OR 'sodium fluoride':ab,ti OR 'calcium phosphates':ab,ti OR 'calcium phosphate':ab,ti OR 'desensitizing agents':ab,ti OR 'cpp-acp':ab,ti AND [embase]/lim	**#3** [randomized controlled trial]/lim AND [embase]/lim
#1 AND #2 AND #3		

Table 2: *Characteristics of the Included Studies*

Study ID	Study Design [Setting]	Method of Color Assessment	Subjects' Age, Mean ± SD [Range] (y)	No. of Subjects Male [%]	Groups/Materials
Browning and others (2008)[35]	Parallel [NR]	Vita Classical[a]	NR	NR	AHD1: 10% CP[b] AHD2: 10% CP[b] + 3% potassium nitrate[c] AHD3: 10% CP[b] + 0.5% potassium nitrate[c] AHD4: 10% CP[d] + 0.5% potassium nitrate + 0.25 sodium fluoride [c] AH5: placebo (without peroxide and desensitizing)[c]
Da Costa and others (2012)[22]	Split-mouth [NR]	Vita Bleached guide[e] and Spectrophotometer Vita Easyshade[f]	NR [21–75]	12 [50%]	AHD: 35% CP in a tray with potassium nitrate and sodium fluoride[g] AH: 14% HP without desensitizer[h]
Alonso de la Peña (2006)[79]	Split mouth [NR]	Vita Lummin Vacuum[i]	31.8 ± 4.5 [18–50]	5 [32%]	AHD: 3.5% HP with a 5% potassium nitrate [j] AH: 10% CP without desensitizer[b]
Gallo and others (2009)[36]	Parallel [NR]	Trubyte Bioform Color Ordered Shade Guide System[k]	NR	NR	AHD: 30% CP with 5% potassium nitrate[c] AH: 30% CP without desensitizer[c]
Giniger and others (2005)[38,92]	Parallel [NR]	Vita Classical[a]	AHD: 44.8 ± 19.0 [NR] AH: 43.6 ± 19.2 [NR] AHD: 43.6 ± NR [NR] AH: 49.2 ± NR [NR]	AHD: 12 [48%] AH: 13 [52%] AHD: 9 [64%] AH: 6 [46%]	AHD: 16% CP with 0.5% soluble calcium phosphate derived in part from calcium nitrate and potassium[l] AH: 16% CP without desensitizer[m]
Navarra and others (2014)[14]	Parallel [university]	Spectrophotometer Vita Easyshade[f]	25.3 ± NR [20–50]	NR [NR]	AHD: 10% CP with potassium nitrate and sodium fluoride[c] AH: 10% CP[c]
Tam (2001)[39]	Split mouth [university]	Vita Lummin Vacuum[i] and photography	31 ± 1 [20–53]	7 [41%]	AHD: 10%CP + with 3% potassium nitrate and 0.11% sodium fluoride[n] AH: 10% CP°
Ziebolz and others (2007)[78]	Parallel [NR]	Adobe Photoshop (L*a*b* values)[p] colorimeter[q]	AHD: 26.6 ± 4.5 [20–48] AH: 26.9 ± 5.3 [20–47]	AHD: 10 [33%] AH: 12 [40%]	AHD: 20% CP with 5% potassium nitrate and sodium fluoride[r] AH: 7.5% HP without desensitizing[s]

Color change was evaluated in five studies using shade guides[22,35,36,38,79] and two studies using objective color measure instruments (spectrophotometer or colorimeter).[14,22] Adobe Photoshop (L*a*b* values) was used in one study[78] and photography in another one.[39]

The number of patients per group in these studies ranged from 16 to 60. The mean age of all participants in the clinical trials was approximately 32.1 years.[14,38,39,78,79] In three of the eight articles, most of the participants were female.[39,78,79] Two studies had a similar sex distribution,[22,38] and in three studies, this information was not reported.[14,35,36]

When considering the bleaching protocol, all studies performed at-home bleaching. In five of seven studies, only CP was used,[14,35,36,38,39] and the remaining used CP and HP in corresponding concentrations,[22,78,79] Concentrations of the bleaching products varied from 10% to 35% for CP and 3.5% to 14% for HP. The time of usage also varied. CP products were used from 30 min to overnight, whereas HP was used from 30 minutes to 3 hours, once or twice daily. The total number of days the patients used the gel varied from 12 days to 4 weeks.

Assessment of the Risk of Bias

A few full-text studies reported the method of randomization and allocation concealment. Blinding was reported in six studies.[14,22,35,36,38,39] At the study level, all eight studies were judged as having an unclear risk of bias, as at least one key domain had an unclear risk (Figure 2).

Meta-Analysis

Meta-analysis was performed on all studies from which the information was reported and could be extracted. This resulted in a different number of studies included in each meta-analysis as described below.

Table 2: *Characteristics of the Included Studies (ext.)*

Study ID	Bleaching Protocol	Bleaching Tray	Mechanism of Action of the Desensitizer	Outcomes Evaluated		
				Color Change	Tooth Sensitivity	Gingival Irritation
Browning and others (2008)[35]	Overnight, Minimum 6 h per 2 wk	With reservoirs	Potassium nitrate: nerve desensitization Sodium fluoride: remineralizing	ΔSGU	Risk of TS without stimulus Total number of days with TS	GI, tongue and throat sensitivity
Da Costa and others (2012)[22]	30 min per 15 d	With reservoirs	Potassium nitrate: nerve desensitization Sodium fluoride: remineralizing	ΔSGU and ΔE	VAS 1–10 without stimulus	NR
Alonso de la Peña (2006)[79]	Once daily for 3 h/d for 4 wk	NR	Potassium nitrate: nerve desensitization	ΔSGU	NRS 0–4 without stimulus	Risk of GI
Gallo and others (2009)[36]	1 h/d for 10 d for a total of 10 h of treatment.	Without reservoirs	Potassium nitrate: nerve desensitization	Final SGU	VAS 1–10 with stimulus.	Löe and Silness Gingival Index
Giniger and others (2005)[38,92]	Once daily for a minimum of 3 h (or overnight) over the course of 14 d	Without reservoirs	Potassium nitrate: nerve desensitization CP: remineralizing	ΔSGU	VAS 0–10 and NRS 0–3 with stimulus	Löe and Silness Gingival Index
Navarra and others (2014)[14]	14 nights at last 6 h	NR	Potassium nitrate: nerve desensitization Sodium fluoride: remineralizing	ΔE	NRS 0–3 without stimulus	NR
Tam (2001)[39]	Overnight for 14 consecutive nights	NR	Potassium nitrate: nerve desensitization Sodium fluoride: remineralizing	VAS color	VAS 0–10 without stimulus	NR
Ziebolz and others (2007)[78]	AHD: Once daily, 3–4 h for 12 d AH: Twice a day, 30 min, for 12 d	With reservoirs	Potassium nitrate: nerve desensitization Sodium fluoride: remineralizing	L*a*b* values	VAS 0–10 with and without stimulus	Oral examination

*Abbreviations: AH: at-home dental bleaching without desensitizer; AHD, at-home dental bleaching with desensitizing; CP, carbamide peroxide; ΔE, color difference measured with a spectrophotometer; GI, gingival irritation (on a 0–3 scale: 0 = healthy tissue and 3 = the most severe gingivitis, including tissues that spontaneously bleed; HP, hydrogen peroxide; ID, identification; L*a*b* values, L*: brightness—dark to light, a*: green to red and b*: blue to yellow; NR , not reported; NRS, numeric rating scale; SD, standard deviation; SGU, shade guide unit; ΔSGU, color change in shade guide units; VAS, visual analog scale; VAS color, visual analog score for tooth color.*

[a] *Vitapan Classical, Vita Zahnfabrik,Bad Säckingen, Germany.*
[b] *10% CP Opalescence, Ultradent Products, South Jordan, UT, USA.*
[c] *Uninformed, brand was not identified.*
[d] *10% CP Opalescence PF, Ultradent Products, South Jordan, UT, USA.*
[e] *Vita Bleachedguide 3D-Master, Vita Zahnfabrik, Bad Sackingen, Germany.*
[f] *Spectrophotometer Vita Easyshade, Vita Easyshade, Vident, Brea, CA, USA.*
[g] *35% CP Opalescence PF, Ultradent Products, South Jordan, UT, USA.*
[h] *14% HP Crest, Whitestrips, Procter & Gamble, Manson, OH, USA;*
[i] *Vita Lummin Vacuum, Vita Zahnfabrik, Bad Säckingen, Germany.*
[j] *3.5% HP with 5% potassium nitrate, FKD, Kin Lab, Barcelona, Spain.*
[k] *Trubyte Bioform Color Ordered Shadeguide system, Dentsply Trubyte, York, PA, USA.*
[l] *The 16% CP gel (NiteWhite Excel 3 Regular) was incorporate the calcium ion (as CaNO3) and the phosphate ion (as K4P2O7) produced exclusively for this study by Discus Dental, Culver City, CA, USA.*
[m] *NiteWhite Excel 3 Regular 16% CP, Discus Dental, Culver City, CA, USA.*
[n] *The 10% CP gel with 3% potassium nitrate + 0.11 fluoride was produced exclusively for this study by Ultradent Products, South Jordan, UT, USA;*
[°] *The 10% CP gel was produced exclusively for this study by Ultradent Products, South Jordan, UT, USA.*
[p] *Adobe Photoshop®, Adobe Systems Inc, San Jose, CA, USA.*
[q] *Dental colourimeter Shade Eye—Shofu Dental Corporation, San Marcos, CA, USA.*
[r] *20% CP Opalescence PF, Ultradent Products, South Jordan, UT, USA.*
[s] *7.5% HP Visalys Whitening, Kettenbach, Eschenburg, Germany.*

Risk of Tooth Sensitivity—This outcome was reported in four studies.[22,35,78,79] The risk ratio was 0.99 with a 95% CI of 0.74 to 1.33 (p=0.95). Data were not heterogeneous (χ^2 test: p=0.74; I^2=0%; Figure 3), which means all studies included in the analysis shared a common effect size.

Intensity of Tooth Sensitivity—This analysis was based on three studies that reported this outcome

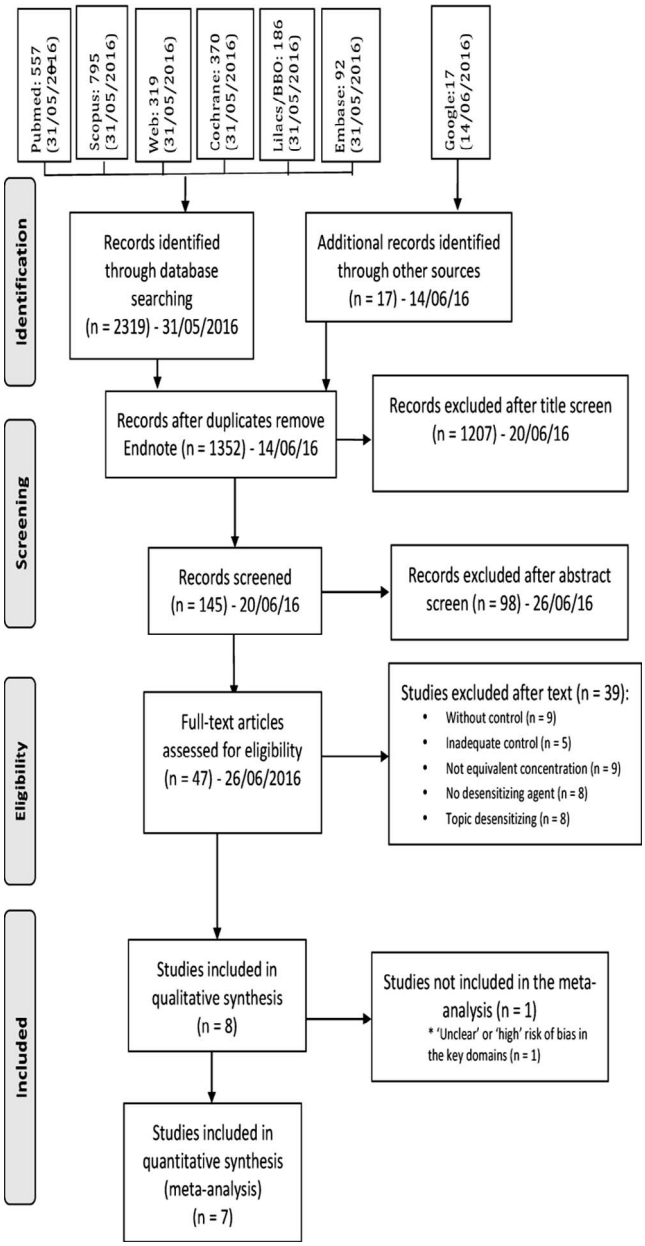

Figure 1. *Flow diagram of study identification.*

with a stimulus.[36,38,78] Although Navarra and others[14] reported the data of TS intensity, their values were not combined with the other studies in the meta-analysis because it was the only study that did not collect data with a stimulus. The standardized difference in means was –0.04 (95% CI=–0.79 to 0.70), with no significant difference in the intensity of TS between the products (p=0.91; Figure 4). Data were heterogeneous (χ^2 test: p=0.006; I^2=81%; Figure 4).

Color Change in ΔSGU—This analysis was based on four studies that reported this information.[22,35,36,38] The standardized difference in means was –0.04 (95% CI=–0.50 to 0.42), which demonstrated no difference in color change between groups (p=0.87; Figure 5). Data were heterogeneous (χ^2 test: p=0.05; I^2=61%; Figure 5).

*Color Change in ΔE**—This analysis was based on only two studies that reported relevant data.[14,22] The standardized difference in means was 0.41 (95% CI=–0.07 to 0.89), showing no difference between the groups (p=0.10; Figure 6). Data were heterogeneous (χ^2 test: p=0.60; I^2=0%; Figure 6).

Risk of Gingival Irritation—This analysis was based on four studies that reported this information.[22,35,78,79] The risk ratio was 1.05 (95% CI=0.81 to 1.36), showing no significant difference between the groups (p=0.95; Figure 7). Data were not heterogeneous (χ^2 test: p=0.74; I^2=0%; Figure 7).

Sensitivity Analysis—The study of Browning and others[35] did not report the standard deviation (SD) of the mean for the ΔSGU, and therefore an SD that corresponded to 1/3 of the mean was arbitrarily used. This decision was based on the analysis of the coefficient of variation of the other studies. Sensitivity analysis indicated that changing the SD for extreme values and removing the study from the meta-analysis would not alter the conclusions.

A sensitivity analysis was performed to identify any study that was responsible for the high heterogeneity in the meta-analysis of the intensity of TS and color change for both instruments. No study was found to be responsible for such heterogeneity.

Assessment of the Quality of Evidence

In the summary of findings (Table 3), the outcomes, other than the risk of TS, graded as moderate in the quality of evidence, were assessed as low and very low quality using GRADE. The reasons for downgrading the evidence were due to the unclear risk of bias of most studies, imprecision due to low sample sizes and high confidence intervals, and inconsistency with an unexplained heterogeneity (Table 3).

DISCUSSION

All studies from the present investigation were judged as having an unclear risk of bias, as most of the authors did not adequately report the sequence generation, allocation concealment, or blinding. This is not a new problem; a study by Devereaux and others[80] reported that 55% of the full-text studies failed to report the presence or absence of the concealment of randomization. In another study,

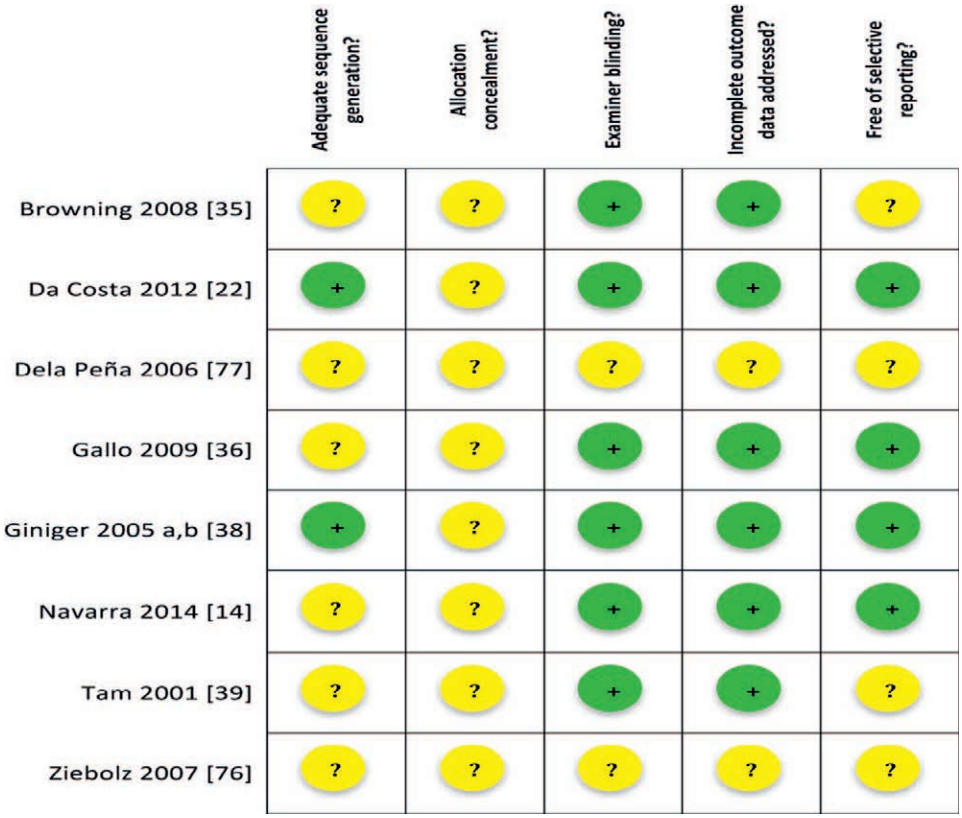

Figure 2. *Summary of the risk of bias assessment according to the Cochrane Collaboration tool.*

the authors reported that the odds ratios were exaggerated by 41% for inadequately concealed trials and by 30% for unclearly concealed trials after adjustment for other variables.[81] The absence of adequate allocation concealment can lead to selection bias, one of the most important problems that randomization is supposed to eliminate.[82] As a result, one may expect an overestimation of the results in benefit of the experimental group being tested for studies with this issue.[81,83]

There is abundant evidence to support that adequate sequence generation, allocation concealment, and blinding are essential for gathering reliable results in clinical trials.[81] A meta-analysis will never produce more trustworthy findings than those of the primary articles. Studies in this field should be more rigorous during study design and the reporting of findings.

When considering the meta-analysis of the risk and intensity of TS, no significant difference between the groups was observed. Few studies were used in this meta-analysis, which restricts the precision of the effect estimate of TS intensity (risk ratio). This lack of difference was attributed to several factors; for example, it is expected that lower TS intensity in the control group makes it difficult to find differences between groups. In the present study it is possible to show that, on a 0–10 pain scale, the intensity of bleaching-induced TS for at-home bleaching was low and ranged for 1.1 to 3.6.[36,38,78] However, the most important factor is the great variability in bleaching protocols reported in the results section. Observe that, while Giniger and others[38] showed favorable results in the desensitizer group, on the other side, Ziebolz and others[78] showed the opposite. Therefore, more RCTs need to be done comparing at-home bleaching techniques using gels with and without desensitizers.

It is worth mentioning that the different types of desensitizing agents incorporated in the bleaching gels could have contributed to the lack of differences. The primary studies used bleaching gels containing only potassium nitrate,[36,79] a combination of potassium nitrate and calcium,[38] or potassium nitrate and sodium fluoride.[14,22,35,39,78]

Note that the action mechanism of the different types of desensitizing agents incorporated in the bleaching gels are not the same. While potassium salts have a presumptive effect on reducing activation of the sensory nerve by preventing the depolarization of the nerve fiber,[84,85]; fluoride and calcium remineralize exposed dentin tubules and, conse-

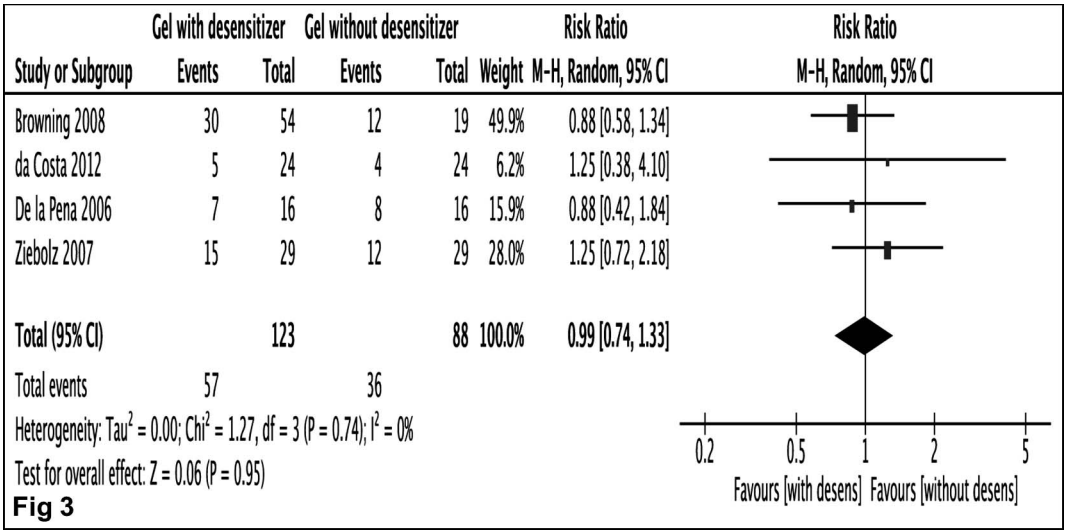

Fig 3

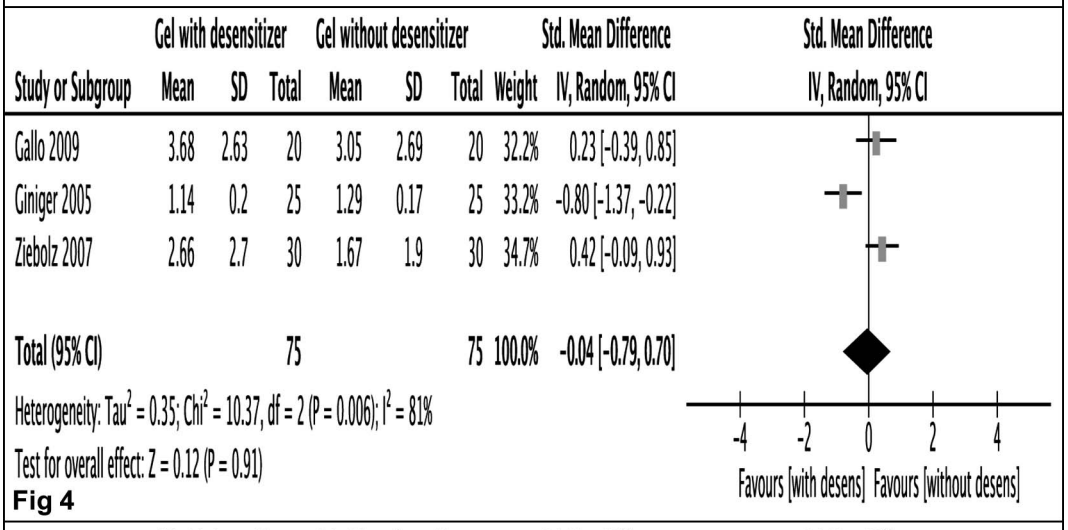

Fig 4

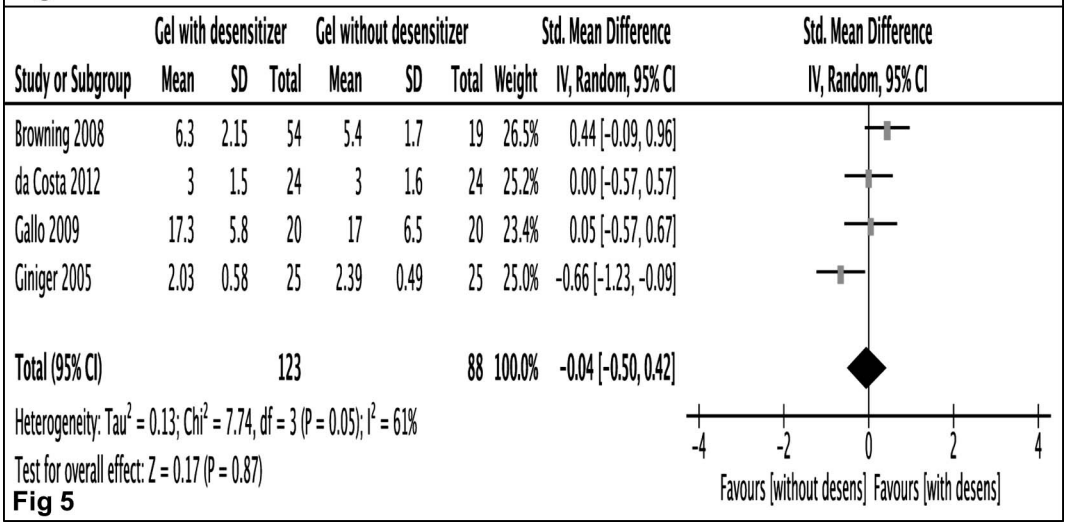

Fig 5

Figure 3. *Forest plot of the risk of tooth sensitivity for dental bleaching with desensitizer vs without desensitizer.*

Figure 4. *Forest plot of the intensity of tooth sensitivity (with stimulus) for dental bleaching with desensitizer vs without desensitizer.*

Figure 5. *Forest plot of the color change in shade guide units for dental bleaching with desensitizing vs without desensitizing.*

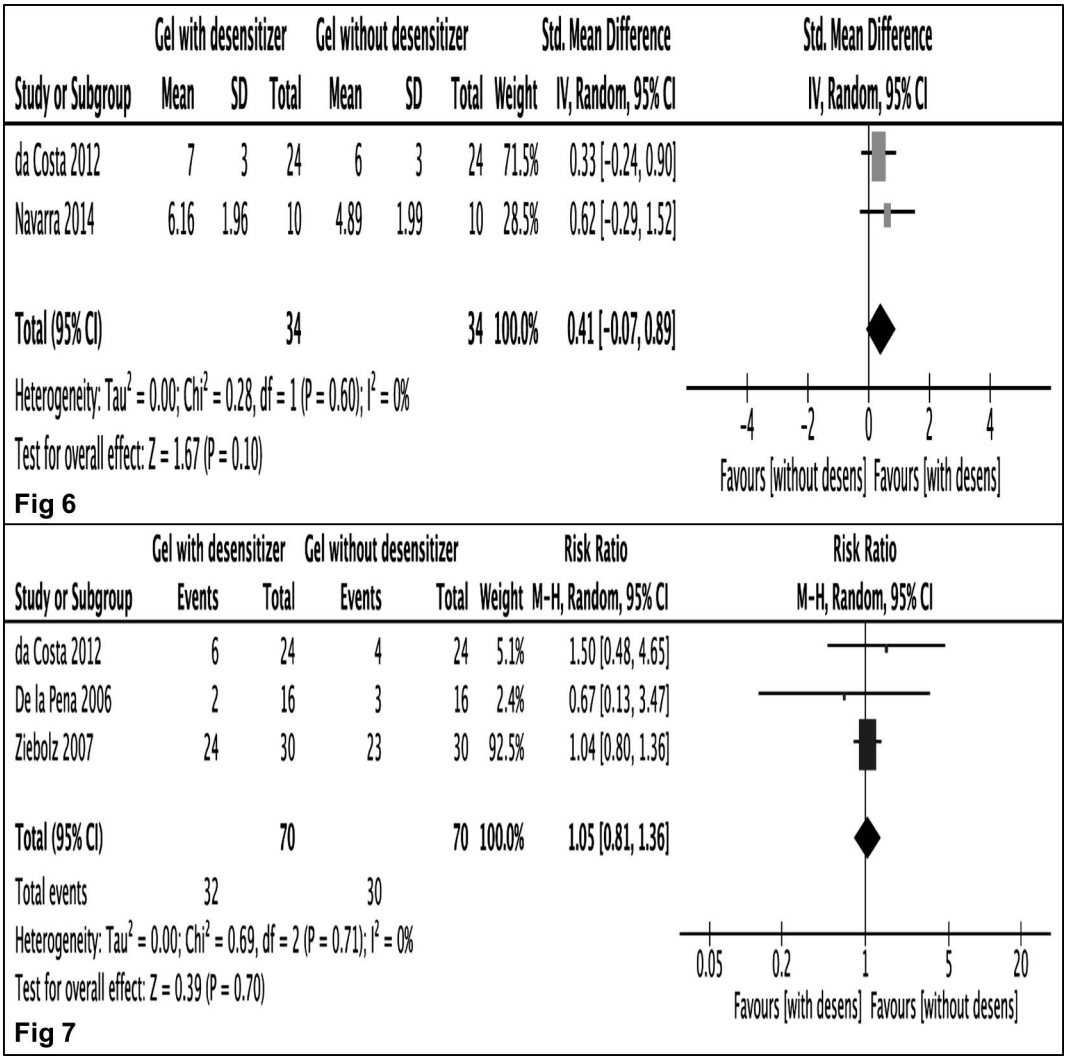

Figure 6. *Forest plot of the color change in ΔE* dental bleaching with desensitizer vs without desensitizer.*

Figure 7. *Forest plot of the risk of gingival irritation for dental bleaching with desensitizer vs without desensitizer.*

quently, reduce dentin permeability.[85,86] Unfortunately, based on the studies included in this systematic review, we cannot affirm the superiority of either treatment approach because in these studies the desensitizing agents were usually mixed in the same product as reported in Table 2. Future RCTs need to be done comparing at-home bleaching gels with and without each specific desensitizer.

Although these desensitizing components are effective, they may need some time to reach the pulp and exert their effectiveness before the application of HP.[27,31,39] The topical application of desensitizing agents appears to be an effective strategy for reducing TS with at-home bleaching[16,26,30] and in-office bleaching.[31-34] As both components are applied simultaneously, HP can reach the pulp tissue faster due to its lower molecular mass.[87] Therefore, by the time potassium nitrate reaches the pulp, reducing transmission of nerve impulses by potassium nitrate[31,79] is no longer possible, as HP may have caused cell damage or even directly activated the neuronal receptors (TRPA1 ion channel) of the dental pulp complex[88] that ultimately triggers the transmission of pain impulses.

All of the included studies used the at-home bleaching technique and reported a color change of three to eight units on the Vita shade scale, which is in agreement with earlier reports in the literature.[4,5,7,10,89] This variation in the number of whitening units can be the result of the different contact periods of the bleaching agent. The effec-

Table 3: *Summary of Findings Table*

Desensitizing-containing compared to desensitizing-free products for adults submitted to dental bleaching						
Patient or population: adults submitted to dental bleaching						
Setting: cosmetic care in university						
Intervention: desensitizing-containing products						
Comparison: desensitizing-free products						
Outcomes	**Anticipated Absolute Effects[a] (95% CI)**		**Relative Effect (95% CI)**	**No of Participants (Studies)**	**Quality of the Evidence (GRADE)[b]**	**Comments**
	Risk With Desensitizer-Free Products	**Risk With Desensitizer-Containing Products**				
Risk of tooth sensitivity assessed with pain scales	409 per 1000	405 per 1000 (303–544)	RR 0.99 (0.74–1.33)	211 (4 RCTs)	⊕⊕⊕○ Moderate[c]	From a total of seven included studies only four reported this outcome.
Intensity of tooth sensitivity assessed with pain scales after stimulation	-	SMD 0.04 SD lower (0.79 lower to 0.7 higher)	-	150 (3 RCTs)	⊕○○○ Very low[c,d,e]	From a total of eight included studies, only three reported this outcome in a similar manner, that is, pain. Measurement was performed after application of stimulus. Only one study (not included) measured pain intensity without stimulation
Color change in shade guide units assessed with shade guides	-	SMD 0.04 SD lower (0.50 lower to 0.52 higher)	-	211 (4 RCTs)	⊕○○○ Very low[c,d,e]	From a total of eight included studies, only four reported this outcome.
Color change in ΔE^* assessed with spectrophotometer	-	SMD 0.41 SD lower (0.07 lower to 0.89 higher)	-	68 (2 RCTs)	⊕⊕○○ Low[c,f]	From a total of right included studies only two reported this outcome.
Risk of gingival irritation assessed with pain scales	429 per 1000	450 per 1000 (347–583)	RR 0.99 (0.74–1.33)	211 (4 RCTs)	⊕⊕○○ Low[c,e]	From a total of eight included studies only three reported this outcome.

Abbreviations: CI, confidence interval; RCT, randomized clinical trial; RR, risk ratio; SD, standard deviation; SMD, standardized mean difference; RCT, randomized clinical trial;.
[a] The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
[b] GRADE (Grading of Recommendations: Assessment, Development and Evaluation) uses the following levels of evidence: ⊕⊕⊕⊕ = high quality means researchers are very confident that the true effect lies close to that of the estimate of the effect; ⊕⊕⊕○ = moderate quality means the researchers are moderately confident in the effect estimate (ie, the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different); ⊕⊕○○ = low quality means researchers' confidence in the effect estimate is limited (ie, the true effect may be substantially different from the estimate of the effect); and ⊕○○○ = very low quality means researchers have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.
[c] The great majority of the studies were at unclear risk in sequence generation and allocation concealment domains.
[d] Inconsistency of the data with unexplained heterogeneity.
[e] High 95% CI, which does not exclude important harm or benefit.
[f] Very low sample size, excluding the optimal information size, with a very high 95% CI.

tiveness of bleaching is not only related to the concentration but also to usage time.[9,23-25,58,90,91] Indeed, by looking at the findings of the primary studies, this difference in the bleaching protocol can be detected. The studies of Costa and others[22] and Gallo and others[36] reported a color change of only three units on the Vita shade guide, whereas the studies of Browning and others[35] and Giniger and others[38] found a variation of approximately eight shade guide units. Although da Costa and others[22] and Gallo and others[36] kept the product in contact with the teeth for 30 minutes to 1 hour, Browning and others[35] and Giniger and others[38] left the gel on the dental surfaces for a minimum of 3 hours.

No difference was found between gels with and without desensitizers when considering gingival sensitivity, which was already expected because no component in the bleaching gel is able to neutralize the action of peroxides in the soft tissues.

In summary, the hypothesis of equality between materials in relation to TS cannot be rejected. This is the only outcome for which the quality of the evidence was considered moderate. However, one

should keep in mind that the quality of the evidence of the outcomes for color change, intensity of TS, and risk of gingival irritation was graded as low and very low. This downgrade in the level of evidence for these outcomes was the result of the low number of studies due to the lack of information in primary studies. For instance, Table 3 explains that only three of eight studies reported the intensity of TS in a similar manner, and only two of eight studies reported color change in delta E. This reduced the total number of participants in the meta-analysis, generating high confidence intervals, with the estimates already coming from studies with an unclear risk of bias.

In light of this, more RCTs should be conducted using a rigorous methodology for at-home bleaching techniques using gels with and without desensitizers. Although the current research strategy included at-home and in-office bleaching, only at-home bleaching studies were found and included in the present systematic review of the literature, indicating that future RCTs need to be performed comparing in-office bleaching techniques using gels with and without desensitizers.

CONCLUSION

Incorporating desensitizers in at-home bleaching gels did not reduce the risk of TS; the quality of the evidence for this outcome was considered moderate. No difference between the groups was observed for the other outcomes (intensity of TS, color change, and risk of gingival irritation), although the quality of the evidence of these outcomes was low or very low. This indicates that more RCTs with large sample sizes and a rigorous methodology are required to confirm these results.

Acknowledgement

This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 305588/2014-1 from Brazil.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the State University of Ponta Grossa. This study was registered at the PROSPERO under protocol number CRD 42016036411.

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.

(Accepted 19 March 2018)

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Effect of Argon Plasma Surface Treatment on Bond Strength of Resin Composite Repair

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Clinical Relevance

The use of argon plasma application as a surface treatment did not improve the bond strength of composite repairs. The traditional protocol combining sandblasting, silanization, and the use of a hydrophobic bonding adhesive was the most reliable method.

SUMMARY

Objectives: This study evaluated the effect of argon plasma treatment (PLA) and its combination with sandblasting (SAN), silanization (SIL), and hydrophobic bonding resin (HBR) application on the micro-shear bond strength of water-aged restorative resin composite to a newly placed composite, simulating restoration repair.

Methods and Materials: Forty-five light-cured composite plates (20-mm long × 20-mm wide × 4-mm thick) were fabricated using a hybrid composite and stored at 37°C in distilled water for six months. The aged composite surfaces were treated according to the following experimental groups, varying both treatment and order of application: 1) SAN + SIL + HBR (control), 2) SAN + PLA for 30 seconds + SIL + HBR, 3) SAN + SIL + PLA + HBR, 4) PLA + SIL + HBR, 5) PLA + SIL, 6) PLA + HBR, 7) SIL + PLA + HBR, 8) SIL + PLA, and 9) PLA. After the surface treatments, four fresh resin composite cylinders (1.5-mm high × 1.5-mm diameter) of the same composite were built on each aged composite surface using a silicone mold. After water storage for 24 hours or one year, the specimens were submitted to shear bond strength testing. Data were statistically analyzed by two-way analysis of variance and Tukey's test (5%).

Results: Groups 1, 2, and 4 presented significantly higher bond strength means at 24 hours, although group 4 did not differ from group 7. Groups 5, 8, and 9 demonstrated significantly lower means than the other groups. Even

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DOI: http://doi.org/10.2341/18-050-L

though groups 1 and 2 had a significant bond strength reduction after 1 year, they still demonstrated higher bond strength at one year of storage.

Conclusions: While PLA application combined with surface treatment methods demonstrated high bond strength results, this treatment alone was not as beneficial as other methods that included SAN, SIL and HBR.

INTRODUCTION

Resin composite restoration repairs are common procedures in general practice. The repair can be performed in both recently placed restorations and old ones.[1-5] In just-placed restorations, the repair is indicated to correct and adjust color, anatomy, lack of margin sealing, or early small composite fractures. In these cases, the prognosis of success is high if the composite restoration is recently placed and unreacted monomers are still available for chemical bonding with the fresh composite increment.[6-8]

Conversely, for older composite restorations, the unreacted monomers are leached out, and there are no chemical bonds available for bonding with a fresh composite. To overcome this lack of chemical bonding between the old and new composites, sandblasting with aluminum oxide has been recommended to increase the superficial roughness and create micromechanical retention at the surface of the old resin composite.[9-11] In addition, silane application is recommended to bond the monomers from the fluid adhesive resin layer and composite to the exposed glass filler of the old composite through a chemical reaction.[9-17]

Atmospheric pressure plasma (PLA) has been used to chemically destabilize surfaces and bond to another matter. The PLA is formed by reactive species that are created by the interaction between ions and electrons of argon PLA; thus, when the PLA micro-atmosphere reaches the sample surface, the reactive species breaks the stabilized bonds and forms polar groups at the surface. In summary, PLA improves the reactive level of surfaces by opening up the chemical sites to future bonds.[18-22]

Argon PLA applied as a surface treatment of old composites may have the ability to enhance the composite restoration repair and improve its longevity.[1,4] Heretofore, no study has been performed to investigate advantages in using argon PLA for resin composite repair. The purpose of this study was to evaluate the effect of argon PLA and its combination with sandblasting (SAN), silanization (SIL), and hydrophobic bonding resin (HBR) treatments on the micro-shear bond strength of water-aged restorative resin composite to a newly placed composite, simulating restoration repair, after 24 hours and one year of storage. The hypothesis tested was that PLA used as a surface treatment would improve the bond strength of repaired composites.

METHODS AND MATERIALS

Specimen Preparation

Forty-five composite (Charisma, Heraeus Kulzer, Germany, shade A2, lot No. 010611) plates (20-mm long × 20-mm wide × 4-mm thick) were fabricated using silicon molds and light activated with a polywave light-curing unit (886 mW/cm^2 irradiance) for 40 seconds (Valo, Ultradent Product Inc, South Jordan, UT, USA). The composite plates were kept in distilled water at 37°C for six months. After aging, samples were polished with 600-grit silicon carbide paper (Norton, Vinhedo, SP, Brazil) and submitted to ultrasonic cleaning (USC 1400, Unique Ind Com Prod Eletr Ltda, Indaiatuba, SP, Brazil) with distilled water for five minutes.

Experimental Groups

Composite plates were divided into nine groups (n=5), varying surface treatments and application order, as described in Table 1. The PLA equipment used was the Surface Plasma Tool Model SAP–Lab applications (Surface-Engineering and Plasma Solution LTDA, Campinas, SP, Brazil). Application time of the argon PLA was 30 seconds as the working gas (Praxair 4.8, White Martins Gases Ind SA, Rio de Janeiro, RJ, Brazil), with an output of 1.0 L/min.

Groups that were sandblasted received the following protocol: air abrasion with 50 μm aluminum oxide particles using a sandblasting unit (Microetcher, Danville Materials, San Ramon, CA, USA) for 10 seconds, 10 mm away from the surface at 60 psi. Afterward, the plates were submitted to an ultrasonic bath (Unique Ind Com Prod Eletr Ltda) in distilled water for five minutes, followed by uniform air drying of the samples using an air syringe for 30 seconds.

The silane-coupling agent used was the Ceramic Primer (lot No. N522201, 3M ESPE, St Paul, MN, USA), and the HBR was the adhesive (Adper Scotchbond Multi-Purpose, lot No., N5154423M ESPE). A drop of the silane was deposited in a mixing well, from which the silane was collected by a micro-brush for application on the composite plates. A uniform layer of liquid silane was applied to the

Table 1: *Experimental Groups According to Surface Treatment and Order of Application*

Group	Treatment
1	SAN + SIL + HBR (control)
2	SAN + PLA + SIL + HBR
3	SAN + SIL + PLA + HBR
4	PLA + SIL + HBR
5	PLA + SIL
6	PLA + HBR
7	SIL + PLA + HBR
8	SIL + PLA
9	PLA

Abbreviations: PLA, plasma treatment; SAN, sandblasting; SIL, silanization; HBR, hydrophobic bonding resin.

plates, which formed a thin layer of the silane on the surface of the sample after drying and evaporation of water and other solvents. Silane was applied, kept undisturbed for 15 seconds, and air dried for 10 seconds. HBR application consisted of a uniform coating of adhesive applied with a micro-brush, followed by light activation for 10 seconds.

Micro-shear Bond Strength Test

Silicone molds (Aquasil Ultra Putty, Dentsply Caulk, Milford, DE, USA) were positioned over the treated, aged composite plate, and the fresh composite (same commercial composite and lot number) was inserted into the mold (1.5-mm high × 1.5-mm diameter). The composite was light activated for 20 seconds, and the mold was carefully removed. Four composite cylinders were manufactured onto each plate. Samples were stored in water at 37°C for 24 hours or one year.

For micro-shear bond strength testing, each composite plate was placed with cyanoacrylate glue (Model Repair II Blue, Sankin Industry Co, Tokyo, Japan) to a jig attached to a universal testing machine (EZ Test, Shimadzu Corp, Kyoto, Japan) and subjected to a bond strength test at a crosshead speed of 0.5 mm/min. Two resin cylinders of each plate were tested after 24 hours and the two remaining after one year. The shear load was applied at the base of the resin cylinders with a loop wire (0.2-mm diameter). Bond strength data were calculated using the peak of loading failure divided by specimen surface area, and means were obtained in MPa. A single failure stress value for each composite plate was calculated by averaging the values of the two resin cylinders from the same plate and evaluation time. Bond strength data were expressed in MPa and statistically analyzed by two-way analysis of variance (ANOVA; repeated-measures approach) and Tukey post hoc test (with a preset alpha of 0.05).

The fractured surfaces were gold coated (MED 010, Balzers, Balzer, Liechtenstein) and examined using a scanning electron microscope (JSM-5600LV, JEOL Inc, Tokyo, Japan) at 35× and 200× magnifications (voltage, 15 kV; beam width, 25-30 nm; working distance, 10-20 mm). The failure mode of each specimen was classified as follows: 1) adhesive failure at the old-new composite interface and 2) cohesive failure within old composite.

RESULTS

Bond strength results for 24 hours and one-year storage are presented in Table 2. The two-way ANOVA results indicated that treatment ($p<0.0001$) and evaluation time ($p<0.0001$) factors significantly influenced bond strength values, with significant interaction between the factors

Table 2: *Bond Strength (SD) of New Composite to Old for All Experimental Groups (MPa)*[a]

Group	Treatment	Evaluation Time	
		24 Hours	1 Year
1	SAN + SIL + HBR (control)	32.7 (1.7) Aa	23.0 (3.2) Ba
2	SAN + PLA + SIL + HBR	34.6 (2.8) Aa	20.3 (4.3) Bab
3	SAN + SIL + PLA + HBR	19.7 (4.2) Ac	18.3 (4.0) Aabc
4	PLA + SIL + HBR	30.2 (3.2) Aab	15.9 (2.1) Bbcd
5	PLA + SIL	8.9 (3.2) Ad	5.1 (0.4) Ae
6	PLA + HBR	20.6 (1.7) Ac	13.8 (3.4) Bcd
7	SIL + PLA + HBR	24.1 (1.2) Abc	11.8 (2.3) Bd
8	SIL + PLA	7.0 (0.7) Ad	2.8 (1.8) Ae
9	PLA	4 6 (0.9) Ad	1.8 (0.6) Ae

Abbreviations: PLA, plasma; SAN, sandblasting; SIL, silanization; HBR, hydrophobic bonding resin.
[a] *Means followed by different lowercase letters (column: comparing groups within the same evaluation time) and different uppercase letters (row: comparing evaluating times within the same group) represent significant statistical differences according to Tukey test ($p \leq 0.05$)*

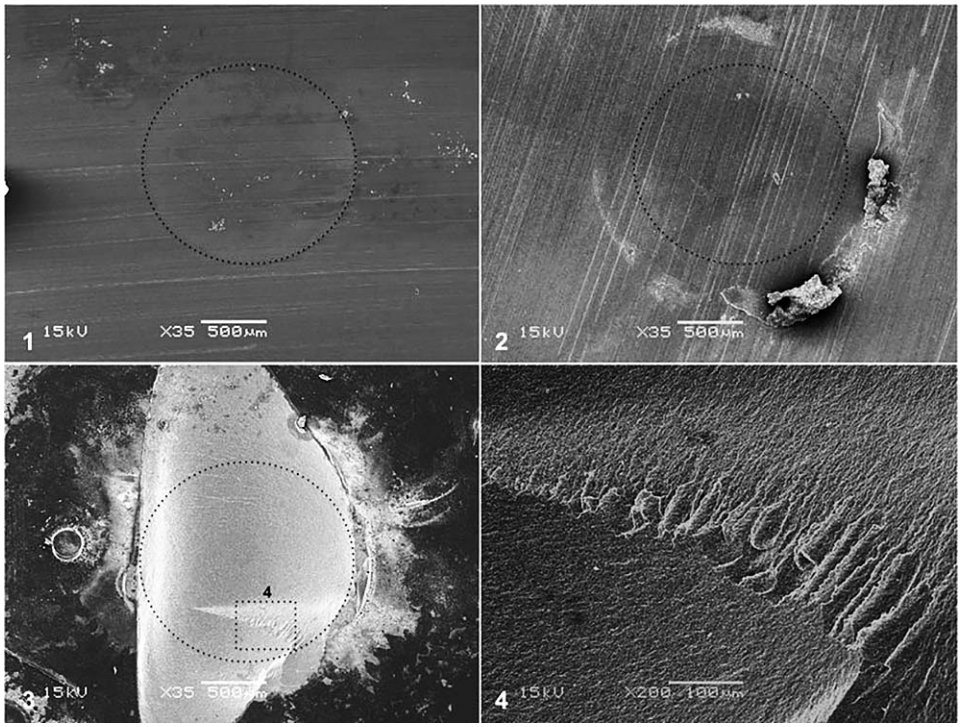

Figure 1. *Scanning electron microscopy photomicrograph illustrating an adhesive failure along the aged composite surface for group 9 (PLA), tested after 24 hours of water storage. Original magnification 35×.*

Figure 2. *Scanning electron microscopy photomicrograph illustrating an adhesive failure along the aged composite surface for group 5 (PLA + SIL), tested after 24 hours of water storage. Original magnification 35×.*

Figure 3. *Scanning electron microscopy photomicrograph illustrating a cohesive failure within the aged composite for group 1, tested after one year of water storage. Original magnification 35×.*

Figure 4. *Higher magnification of Figure 3 showing the aged composite cohesive fracture. Original magnification 200×.*

($p<0.0001$). At 24 hours, groups 1 (control, SAN + SIL + HBR), 2 (SAN + PLA + SIL + HBR), and 4 (PLA + SIL +HBR) presented higher bond strength (between 34.6 and 30.2 MPa), although group 4 did not differ statistically from group 7 (SIL + PLA + HBR). Groups 5 (PLA + SIL), 8 (SIL + PLA), and 9 (PLA), exhibited the lowest bond strength means (between 4.6 and 8.9 MPa), being statistically different from all other groups. Groups 3 (SAN + SIL + PLA + HBR), 6 (PLA + HBR), and 7 exhibited intermediate bond strength values, which were not significantly different from each other.

After 1 year, when storage time is compared, groups 3 and 5 did not demonstrate significant statistical reduction in bond strength. Even though groups 1 and 2 had a significant bond strength reduction after 1 year, they still demonstrated a higher bond strength at one year of water storage (20.3 and 23.0 MPa, respectively). However, group 2 itself did not differ statistically from groups 3 and 4. Groups 5, 8, and 9 maintained significantly lower bond strength means after one year (between 1.8 and 5.1 MPa) compared with the other groups.

Representative images of the failure modes are presented in Figures 1 to 4. Groups 5 (PLA + SIL), 6 (PLA + HBR), and 9 (PLA) had 100% adhesive failures at both composite interfaces, whereas groups 2 (SAN + PLA + SIL + HBR) and 4 (PLA + SIL + HBR) presented 100% cohesive failure within the old composite at both times (Table 3). An increase in adhesive failures was observed for group 7 (SIL + PLA + HBR; from 40% to 100%), whereas increases in cohesive fractures (within the old composite) were noted for groups 3 (SAN + SIL + PLA + HBR / from 40% to 80%) and 8 (SIL + PLA / from 60% to 80%) after one year of storage. The control group (SAN + SIL + HBR) presented 20% adhesive failures and 80% cohesive fractures within the old composite, which did not change after one year.

DISCUSSION

The hypothesis that PLA used as a surface treatment would improve the bond strength of repaired composites was rejected. PLA application alone or combined with SAN, SIL, and HBR did not result in higher bond strength when compared with the control (SAN + SIL + HBR). Nevertheless, the PLA treatment associated with SIL and HBR demonstrated similar results to control at 24 hours, despite the use of SAN or not (groups 2 and 4). Only after one-year of storage was the use of SAN combined with PLA shown to be relevant (group 2), obtaining similar results to the control (group 1).

This gold standard control composite repair technique was used after carrying out a bibliographical survey on this subject, which indicated that the technique and materials used have obtained the best

Table 3: *Failure Modes (%) Among Experimental Groups*

Group	Treatment	Evaluation Time	
		24 Hours AD/CO	1 Year AD/CO
1	SAN + SIL + HBR (control)	20/80	20/80
2	SAN + PLA + SIL + HBR	0/100	0/100
3	SAN + SIL + PLA + HBR	60/40	20/80
4	PLA + SIL + HBR	0/100	0/100
5	PLA + SIL	100/0	100/0
6	PLA + HBR	100/0	100/0
7	SIL + PLA + HBR	40/60	100/0
8	SIL + PLA	40/60	20/80
9	PLA	100/0	100/0

Abbreviations: AD, adhesive failure; CO, cohesive failure; PLA, plasma; SAN, sandblasting; SIL, silanization; HBR, hydrophobic bonding resin.

results.[9-11,14,17,23-25] However, the comparison of the results must be done carefully when the studies have used different types of composites, as well as surface treatment protocols (mechanical and chemical) and materials (conditioners, adhesives, and silanes).[7,8,12,13,15,16,26,27]

To obtain better clinical outcomes, some authors have suggested that knowing the composition of the composite to be repaired is important for the success of the technique. In addition, the different types of composites (microhybrid, nanohybrid, or nanofilled) seem to produce different results according studies.[7,13,15] The presence of fillers in a polymer network can greatly affect water uptake and dissolution, possibly in direct relation to its proportion as it reduces the overall volume of the absorbing polymer.[28] Another factor that is critical to the success of the repair technique is the age of the restoration. Thus, better repair adhesion between the placed restoration and the new composite is found in recently placed restorations.[6,8,11]

In the current study, samples were aged by immersion in distilled water for six months before repairing and testing, which was the same time used by Rathke and others[25] and Celik and others.[12] Artificial aging in water was performed to leach unreacted monomer layer formed due to inhibition by oxygen at the surface of the composite and to simulate oral conditions. Sorption and solubility may serve as precursors to a variety of chemical and physical processes that create biological concerns as well as produce deleterious effects on the structure and function of the dental composite. These effects may include volumetric changes such as swelling, physical changes such as plasticization and softening, and chemical changes such as oxidation and hydrolysis, all processes that help to degrade the material.[28] Other studies have aged the samples for shorter times, such as seven days in saline solution,[16] seven days or one month in water,[8,29] and three months in artificial saliva,[26] while Staxrud and Dahl,[17] in 2015, repaired composite samples that were six years old. Accelerated aging of 300 hours in a weathering tester,[11] 5,000 thermocycles,[13,15] and a combination of thermocycling and water storage also have been used.[27]

The air abrasion promoted by SAN with aluminum oxide particles creates micro retention and a uniformly rough surface, which increases the superficial area that interacts with a bonding agent and the new composite increment.[9,10,14,16,25] According to different studies, in the repair technique, SAN must be used to treat composites, corroborating the results of this study. Actually, for 24-hour testing, the use of SAN was not primordial for achieving high bond strength. Group 4, which used only PLA + SIL + HBR, had similar results to the application of the same protocol using SAN with or without PLA treatment. Nevertheless, after one year of water storage, the group that did not use SAN demonstrated inferior outcomes compared with these same groups. Summarizing the results, when SAN was used in combination with PLA, SIL, and HBR, regardless of the sequence of the use of PLA, SIL, and HBR, higher bond strengths were observed after one year. This behavior demonstrates that the chemical bonding created by PLA and SIL was initially adequate, but after hydrolytic degradation of this interface, the mechanical approach of SAN was necessary. However, in consideration of the safety of the patient and professionals during intraoral sandblasting, an aspirator device and rubber dam isolation must be used, which limits its clinical use.[30] As an alternative, rotary instruments may also be able to roughen the old composite restoration surface, and studies have shown significant improvement in bond strength following surface treatment with diamond burs.[11,16]

The argon PLA application proposed in this study modifies the hydrophilicity and the reactivity of the surfaces but does not promote mechanical changes.[18-21] The reactive species produced by PLA with the purpose of enhancing the reactive level of the composite surface was not enough to improve the bond strength when used alone (group 9). The use of PLA in combination with SAN, SIL, and HBR (groups 2 and 4) did not interfere with bond strength results; however, when PLA was used with only SIL and HBR (groups 5 and 6), lower bond strengths

were observed compared with SAN, SIL, and HBR used together. Substituting SAN (group 1) with PLA treatment (group 4) did not generate a significant difference at 24 hours; however, the bond strength was reduced significantly in approximately 50% of group 4 and 25% of the control group after one year; these results were statistically different, as previously discussed.

Because there is a low amount of unreacted monomers in the surface of aged composites, another chemical reaction for creating a chemical bonding mechanism is important for composite repair techniques. Silanes consist of a silanol group (or alkoxy group) that chemically reacts with hydroxyl groups from silica-based filler particles and the methacrylate group that co-polymerizes with the resin matrix of the new composite increment.[31,32] Some studies have indicated use of silane in an attempt to improve the bond strength of the composite repair; however, the silanization efficacy depends on the type of filler particles and the amount silica available at the surface.[9,17,24,33]

The use of bonding agent has been recommended for composite repair especially because when the composite surface is air abraded or roughened, the fluid adhesive resin is able to penetrate into formed micro-porosities. After light activation, the bonding resin remains attached and interlocked to the surface.[6,15] In this study, for groups 5, 8, and 9, in which HBR was not used, the lowest bond strength values were found at both evaluation times. Various studies have recommended the use of a bonding agent[14,16,23,25-27,29,33]; however, they used different types and generations of adhesive systems. More hydrophobic fluid adhesive resins are preferred, because more hydrophilic adhesives tend to result in early degradation of the adhesive layer with decreasing of bond strength even after short-term evaluation.[14]

The long-term storage time of this study was one year, which was fundamental for analyzing the behavior of different treatments over time. The groups that obtained higher bond strength values (between 34.6 and 20.6 MPa) at 24 hours (groups 1, 2, 4, 6, and 7) showed a bond strength reduction after one year. Although groups 2 (SAN + PLA + SIL + HBR) and control (group 1: SAN + SIL + HBR) presented significant bond strength reduction after long-term storage, they still demonstrated higher bond strength at one year (20.3 and 23.0 MPa, respectively). Group 3 (SAN + SIL + PLA + HBR/ 18.3 MPa), for which no bond strength reduction was observed, did not differ from groups 1 and 2 at one year and had the best outcomes in this study. Two other studies that evaluated the influence of surface treatments on repair bond strength of aged composites showed that the use of aluminum oxide sandblasting and adhesive application yielded stable bond strength after six months.[14,16] Conversely, Staxrud and Dahl,[27] in 2011, reported that the thermocycling method used for aging of the samples affected the bond strength of fresh composite to one year of age, which could compromise the durability of composite repair.[17]

Water storage for one year influenced the failure pattern for only three groups (3, 7, and 8), with an increase in the incidence of adhesive or cohesive failures, depending on the experimental group (Table 3). Groups 5, 6, and 9, which were not treated by sandblasting but rather with PLA, showed 100% adhesive failure. The cohesive failures within the aged composite resulted in part from the strong interaction between materials and the micro-shear bond strength test design, which favors this type of fracture at the adherent structure.[34-36]

This study used a shear bond strength test with a circular bonding area of 1.77 mm^2, which is considered a micro-shear method because its bonded area is lower than 3 mm^2 and is more favorable than a "macro-size" one.[37-39] Bond strength tests such as shear and tensile present advantages, disadvantages, and limitations, and no single bond strength methodology provides a strong clinical correlation.[34,39-41] The micro-tensile method is preferred to evaluate the bond strength of adhesive/composite resins in enamel and dentin, but in studies of resin cement adhesion in zirconia, other types of ceramics, indirect resin, as well as composite resin repair studies, the shear test is still used.[7,11,17,27,29,42-44] For the micro-tensile test, the load is more uniformly distributed at the interface, which is the main advantage of this test, whereas some authors have reported that the shear bond strength method generates a complex stress field at the interface, and adhesive failure occurs predominantly as a result of tensile stresses induced by bending moments.[34-41]

Some studies have discussed the teaching, feasibility, and durability of repairs of composite restorations,[2,4,9] and the reported results showed that the repair technique can increase the clinical longevity of the restorations.[3,5] However, others have criticized the durability of composite restoration repairs, following the unsuitable results obtained and the lack of a specific protocol.[1,13,24] As this study also demonstrated a significant bond strength reduction

between new and old composite materials for most of the groups after one year, perhaps additional macroretentions in the cavity preparation may be necessary to ensure longer repaired restoration durability, especially in the cases of older composite restorations.

CONCLUSIONS

Within the limitation of the shear bond strength method, PLA used alone as a surface treatment for resin composite repair was not as beneficial as other protocols that included SAN, SIL, and HBR in combination or not with PLA. When SAN was substituted by PLA as surface treatment, it presented similar outcomes when associated with SIL and HBR after 24 hours of storage; however, after a long storage period, SAN was demonstrated to be essential to the bond strength of repaired resin composites. The bond strength stability of the interface between aged and fresh composite was not determined in this study, at least for the protocols that presented acceptable initial bond strength values.

Acknowledgements

This study was supported by grants from CNPq (307217-2014-0) and CAPES (1777/2014), Brazil.

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.

(Accepted 4 October 2018)

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Amalgam Strength Resistance to Various Contaminants

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Clinical Relevance

This study provides understanding of the potential compromise of amalgam strength after maximum contamination exposure during clinical placement.

SUMMARY

Purpose: The purpose of this study was to quantify the relative strength tolerance of 1-day and 30-day amalgam following saturation contamination with water, saliva, blood, and handpiece lubricant oil during condensation.

Methods and Materials: Valiant PhD XT amalgam was tested with 300 shear-strength (N=15) and 120 compressive-strength (N=6) specimens, divided into 1-day and 30-day groups, each with control, water, saliva, blood, and lubricant oil contamination samples. Shear specimens were condensed in 4 × 4-mm anchor wells inundated with contaminant fluids before adding a ring mold with 3.5-mm-diameter central hole adapted immediately to the top for continued condensation under contaminant-submerged conditions. Compressive specimen samples were condensed while completely inundated by each contaminant using the American Dental Association Specification No. 1 amalgam mold apparatus. All specimens were tested with the Instron E3000 and E10000 at 0.5 mm/min, with data statistically evaluated using the Kruskal-Wallis procedure with IBM SPSS v25 and Wilcoxon signed ranks test.

Results: Shear test values (mean±SD) following intracapsular and extracapsular contamination after 30 days under 100% humidity at 37°C were as follows: control, 30.97±5.41 MPa; water, 30.63 ±4.41 MPa; saliva, 27.54 ±4.56 MPa; blood, 24.92 ±3.48 MPa; lubricant oil, 26.06 ±4.06 MPa. Compressive strengths (±SD) of similarly contaminated samples were as follows: control, 447.7 ±76.3 MPa; water, 343.6 ±70.1 MPa; saliva, 307.7 ±24.0 MPa; blood, 281.6 ±35.2 MPa; lubricant oil, 227.8 ±16.9 MPa.

Conclusions: Saliva, blood, and handpiece oil diminish compressive strength significantly, but water shows no statistically significant effect ($p>0.05$). Amalgam 30-day shear strength is significantly altered by contamination with water, blood, or handpiece lubricant oil ($p<0.05$). Remaining amalgam strength after extensive contamination may still be clinically

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DOI: http://doi.org/10.2341/18-091-L

functional relative to a previous ADA recommendation and when compared with resin-based direct restorative materials.

INTRODUCTION

Amalgam restorative material continues to provide a valuable service to dentistry, with over sixty per cent of US general dentists in a 2017 public health survey reporting the use of dental amalgam in their practice.[1]

Adequate compressive strength is essential for the success of an amalgam restoration and depends to a large degree on factors related to its metallic composition and handling.

The deleterious delayed expansion produced by moisture contamination in low copper zinc-containing amalgam was documented as early as the 1940s.[2-4] When uncontaminated, however, compressive strengths between zinc and non-zinc amalgam alloys were not significantly different.[5] Even when contaminated, zinc-free amalgams showed little or no delayed expansion[3-8] and no appreciable changes in compressive strength.[9]

K. W. Ray investigated the problem of amalgam contamination in 1941 and found that saliva, water, or salt solution incorporated into amalgams made from zinc alloys caused excessive growth and corrosion.[3] Significantly, he noted that the same was not true for amalgam specimens made from non-zinc alloys. Sweeney's laboratory confirmed this observation,[3] and results have been repeatedly confirmed by other investigators.[4,9,10] Data from a classic study by Phillips and others with non-zinc alloys showed no difference in strength at successive time intervals for up to 1 year, whether contaminated or uncontaminated (Table 1).[9]

Historically, when amalgam was triturated with a mortar and pestle, it was common to knead the fresh mass of amalgam in the palm of the bare hand prior to placement into the cavity preparation. This practice was well before mandatory use of rubber gloves, so perspiration from the skin became a contaminating source, estimated by the American Dental Association (ADA) to be equivalent to one drop of salt water.[2] Healey and Phillips in 1949 visually examined 1521 defective amalgam restorations in patients presenting to the Indiana University School of Dentistry operative dentistry clinic. They reported that, of the 1521 defective restorations they personally examined, 16.6% had resulted from delayed expansion.[11]

In all these published investigations, experimental conditions involved contamination that was introduced either before trituration or during palming of the amalgam. An exception was the study by Eames and others[6] that included contamination during condensation. His group condensed amalgam into 5 $\times$ 10-mm saliva-filled Teflon molds. Although saliva is the most common clinical contaminant, a literature search indicated that this 1973 study may be the only one looking at saliva itself as the contaminating source. A concise summary describing the experimental contamination methods of original investigators[2-7,9,10,12,13] is shown in Table 2.

Amalgam strengths prior to 1977 had been analyzed primarily using the diametral tensile test 15 minutes after mixing. In 1977, the ADA Council on Dental Materials and Devices replaced that test method with the one-hour compressive strength test, which was less technique sensitive and more reproducible.[14]

Since the introduction of dispersed-phase high-copper amalgam (HCA) alloys in the 1960s, studies confirmed beyond reasonable doubt that HCAs were not subject to dimensional changes resulting from fluid contamination.[6] In fact, more recently, the presence of zinc in HCAs has been reported as contributing to improved marginal integrity.[15,16]

Dental amalgam has widely received the reputation as a forgiving restorative material regarding its placement when contamination control is questionable. However, no reports were found that quantified the extent to which saturation with various contaminants, either prior to or during condensation, might affect the final strength of non-zinc ($<$0.04%) HCA.[12] Especially in managing clinical cases with less than ideal moisture control, it is useful for practitioners to understand the ranges of strength loss that might be expected should dental amalgam be maximally inundated with liquid contaminants (ie, totally uncontrolled) at placement.

The purpose of this laboratory study was to quantitatively differentiate the strength of water-, saliva-, blood-, and oil-contaminated amalgam samples from uncontaminated controls after 1 day and 30 days.

The null hypotheses for this study were that, relative to the control samples, there would be no statistically significant differences among the compressive or shear strengths of specimens prepared by condensing through various fluid contaminants for a high-copper dispersed-phase non-zinc dental amalgam.

Table 1: *Compressive Strengths of Non-zinc Versus Zinc-Type Amalgams: Thirty-day Compressive Strengths for Non-zinc Alloy in a Classic Study by Phillips and Others*[9]

Amalgam Types	Time Intervals		
	24 Hours	30 Days	60 Days
Non-zinc contaminated	312 (1%)	324 (−8%)	342 (1%)
Non-zinc control	311	351	338
Zinc contaminated	322 (−3%)	263 (−21%)	250 (−28%)
Zinc control	334	333	348

This table shows that zinc-containing amalgam contaminated with three drops of saline prior to condensation can be expected to lose roughly 20% of compressive strength stored at room temperature 30 days and nearly 30% after 60 days. Non-zinc alloys, however, showed no appreciable change in compressive strength at all for time intervals up to 12 months. Values in megapascals. N=6.

METHODS AND MATERIALS

This study involving 420 specimens was designed to assess the relative effects that various contaminants at placement may have on amalgam strength. A flow chart is provided in Figure 1.

Pre-encapsulated amalgam capsules (Valiant PhD-XT Sure-Cap, 800-mg alloy, 3-spill size, Ivoclar Vivadent, Inc, Amherst, NY, USA) were mixed using a triturator (Silamat S5, Manufacturer #002603, Vivadent ETS, Schaan FL, Austria) at 10 seconds per capsule, according to the manufacturer's instructions.

Pre-encapsulated amalgam was purchased directly from the manufacturer. Capsules had laser-sealed lids designed to be snapped off to release the amalgam contents after trituration.

After one drop of each contaminant was introduced inside the amalgam capsules, 40 × 9.4-mm pieces of electrical tape (Scotch Super 33+, Vinyl Electrical Tape, St Paul, MN, USA) were used to reseal the opened amalgam capsule lids with slight overlap of the ends and pressed tightly into the lid joint interface to assure no leakage of capsule contents during trituration. Control amalgam capsules (having no contaminant) in this group were also opened and then similarly resealed (Figures 2a through c).

A programmable timer with audible signal (model 06-662-51, Fisher Scientific, Traceable Calibration Control Company, Rochester, NY, USA) notified when set times had elapsed throughout this study.

Shear Strength Specimen Preparation

Anchor-well cylinders were poured with a mixture of orthodontic acrylic at a volumetric 2:1 powder:liquid ratio (polymethyl methacrylate diethyl phthalate polymer #003-07-0358, monomer #871-06-0719, Esschem Co, Linwood, PA, USA). One-inch (25-mm) diameter cylinders approximately 21 mm in height were prepared in a polyoxymethylene (Delrin) 15 hole specimen mold (Part #1599, Ultradent Products, Inc, South Jordan, UT, USA).

To serve as shear testing amalgam specimen condensation molds, fifteen 3.75 mm-thick Delrin disks were machined to 25 mm (1-inch) diameter with 3.5-mm-diameter centered holes, with each disk receiving a bisecting cut to produce two halves for easy removal from the molded specimen (Moran Innovations, Inc, Redlands, CA, USA).

Flat faces of the solid poured-resin cylinders were model-trimmed perpendicular to their outer walls. Holes to serve as cavity wells for the amalgam were drilled 4 mm deep in the center of the face, using a

Table 2: *Various Modes of Amalgam Contamination Reported in the Literature*

Investigators	Modes of Amalgam Contamination Used
ADA Commission 1941[3]	One drop of saturated NaCl solution was added to the alloy before amalgamation (trituration)
Sweeney 1941[4]	The mix of amalgam was mulled in the bare palm with the addition of one drop of a 0.9% solution of NaCl before condensation
Schoonover and others 1942[5]	One drop of salt water was placed in the capsule prior to trituration
Phillips and others 1954[9]	Mulled for 30 seconds in the palm of the hand with three drops of normal saline solution, prior to condensation
Zaazou, 1967[2]	Two drops of saline were added to the alloy and mercury inside the capsule prior to trituration
Eames and others 1973[6]	Specimens were packed into 5- × 10-mm saliva-filled Teflon molds
Fainsilber and others 1980[7]	20 μL 0.5% NaCl solution was added during trituration
Yamada and others 1981[11]	Amalgam was kneaded in the palm of the hand moistened with 2% saline solution for 20 seconds
Nelson and others 1990[12]	10 μL water was added to the sample during trituration, followed by two seconds of final trituration, which authors considered a "worst case scenario"
Osborne and others 1994[13]	10 μL water was added to the die after the first increment of alloy was placed, before condensation

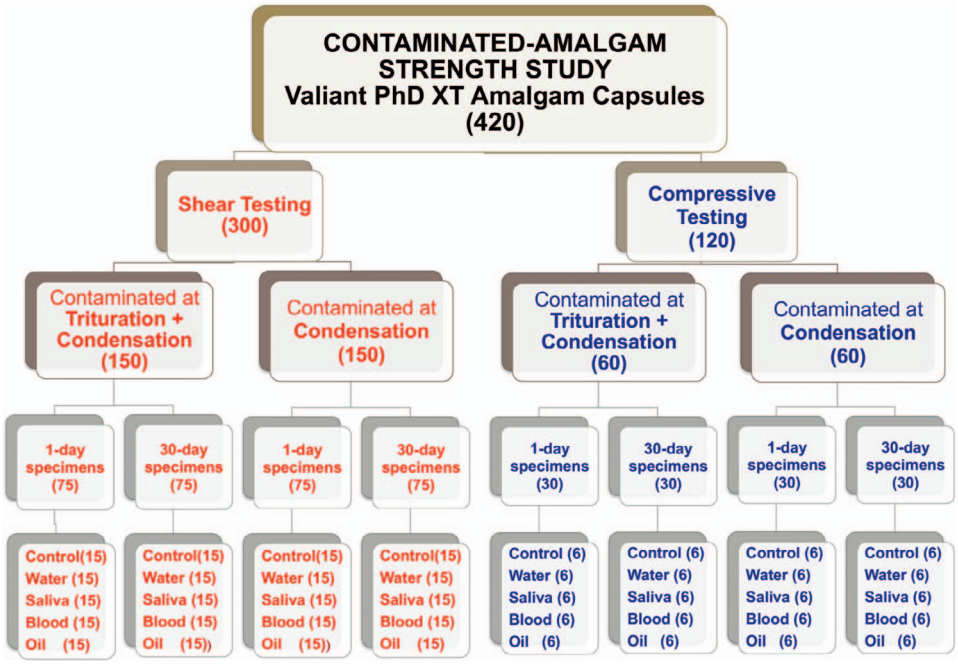


Figure 1. *Flow chart of overall research outline.*

machine lathe with a 4-mm (5/32-inch) diameter drill bit.

Each shear-testing sample group consisted of 15 poured-resin cylinders fully inserted into the 15 holes of the polytetrafluoroethylene molding tray, with 15 Delrin split-ring specimen forming molds (Moran Innovations) snugly seated into the recessed area of the same holes on top of the cylinders, adopted from a previous study testing shear strength of repaired amalgam.[17] Each inserted cylinder top surface was approximately 3-4 mm below the surface of the mold tray, providing space to seat each disk (Figure 3). This tray of specimens was clamped to a flat metal underplate to stabilize the assembly.

A standard amalgam carrier (stainless steel, double-ended; Pulpdent Corporation, Watertown, MA, USA) was adapted to serve as the calibrated amalgam condenser. To standardize the condensation force, the carrier's spring tension was increased by bending it to a tighter curvature. This tension was calibrated on a digital balancing scale (Nexus NS-2000, Polyproducts Corp, Roseville, MN, USA) to a force of 1 lb (4.45 N). To standardize the condensation force, the carrier's spring tension force of 1 lb (16 oz) was measured at the point when the carrier sleeve was depressed.

A special nib was machined from stainless steel (Moran Innovations) to friction-fit over the large end of the amalgam carrier. This had a 1.5 mm-diameter (1.77-mm^2) nib 5 mm long, with a flat, non-serrated tip allowing for condensation of amalgam in the 3.75 mm-deep holes of the split-ring molds. The resulting pressure per condensation stroke with this tip was 2.52 MPa (4.45 N/1.77 mm^2). All shear-strength specimens throughout were condensed using this same calibrated condenser (Figure 4).

Each fresh amalgam mass was sectioned into four pieces using a sharp blade. Two of the quarters were used to fill the anchor-well in the acrylic cylinder below, and the remaining two filled the specimen-forming disk fitted on top.

Before any amalgam was introduced into the anchor well, the well was partially filled with the contaminant appropriate for that part of the study.

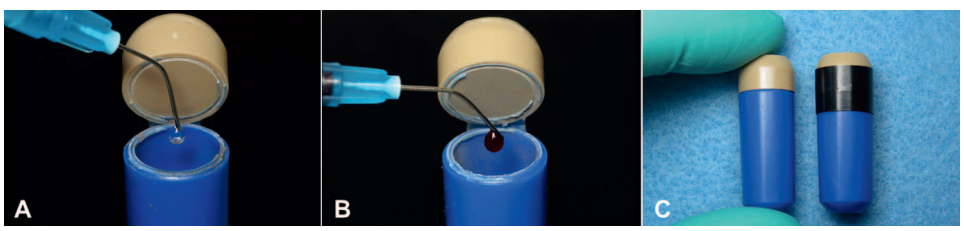
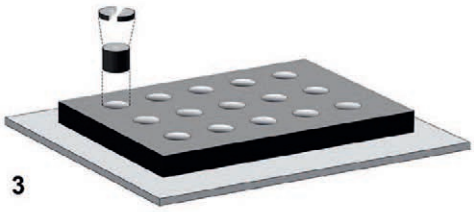
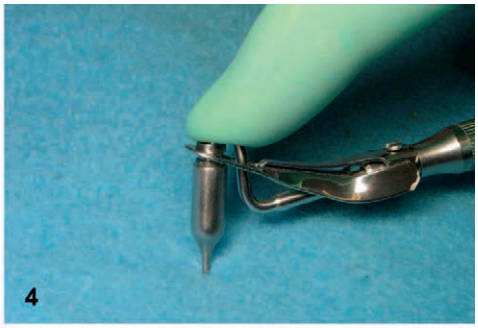


Figure 2. *(a and b) Drops of blood and saliva delivered to the trituration capsules. (c) After one drop of contaminant was placed, the capsule was resealed using vinyl electrical tape to avoid leakage during trituration.*





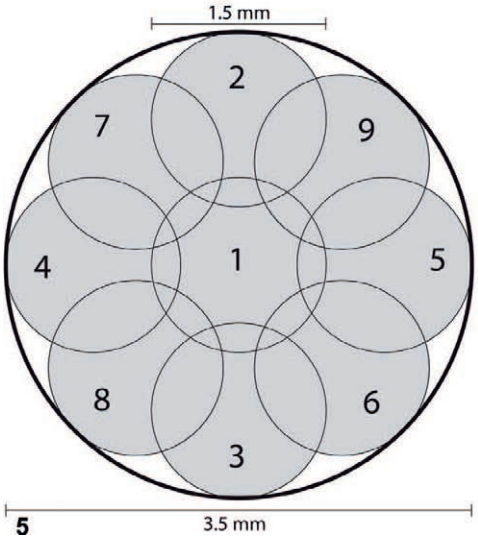


Figure 3. *Diagram of 15-hole mold tray assembly showing how split-ring top molds fit into the holes over the acrylic cylinder specimen anchor blocks.*

Figure 4. *Amalgam carrier modified as a condenser, depressed to the limit of 1 lb-force with custom 1.5 mm-diameter condensing nib attached.*

Figure 5. *Within the 3.5 mm-diameter specimen wells, the 1.5 mm-diameter condenser tip was placed once in the center and eight times around the perimeter to provide thorough condensation.*

Figure 6. *(a and b) Force-calibrated condensation of shear test amalgam through the central hole of the top split-ring specimen-forming disk inundated with saliva and with blood contaminants.*

It was subsequently loaded with the first amalgam increment and lightly directed into place using a 2.5 mm-diameter amalgam condenser (SS #1A, American Dental Mfg Co, Missoula, MT, USA) to approximately 1.5 mm below the top of the resin cylinder before condensing with the calibrated 1 lb-force instrument in a set pattern of nine strokes (Figure 5).

After assuring inundation with fluid contaminant, the next increment of amalgam was condensed in the same nine-stroke pattern using the 1 lb-force (0.45-kg) calibrated condenser. Layers of fluid contaminant were continually delivered to the amalgam surface to keep it flooded (Figure 6).

Sufficient extra amalgam was needed to produce a deliberate excess of mercury-rich layer on top, which was pressed down with a spreading or smearing action with less than 1 lb force using the polished flat handle of the cotton pliers to eliminate inadvertent voids. A razor blade at a low angle was used to shear off this soft minimum 1 mm-deep mercury-rich layer flush with the top of the acrylic cylinder. This surface was critical as the primary test plane between the anchor amalgam below and the form-molded cylindrical 3.5 mm-diameter test column or stub standing above.

Each acrylic resin cylindrical test block was transferred to one of the holes in the 15 hole resin-cylinder mold tray, where a split-ring Delrin disk was inserted and pressed tightly against the acrylic cylinder beneath it. These split-ring molds were a snug friction fit, being 1 inch in diameter, the same as the Ultradent mold holes. When secured into place, the central hole of the top mold was filled with fluid contaminant (either water, saliva, blood, or oil) to assure inundation of the fresh razor-cut amalgam surface located at the floor of this chamber. The central holes were slightly smaller in diameter (3.5 mm) than the anchor well (3.9 mm) to allow for complete contact of the amalgam to the anchor-well below in case of slight lateral eccentric discrepancy. It was vital that the contaminant material cover the entire critical surface.

One of the two smaller cut pieces of amalgam was lightly pushed into the split-ring central hole with the 2.5 mm diameter end of the amalgam condenser (amalgam condenser #1A, American Dental Mfg Co) with less than 1 lb-force. The amount of amalgam inserted was about 2 mm deep (approximately half the thickness of the 3.75 mm Delrin ring). After another layer of liquid contaminant inundated the surface, the nine-stroke pattern was used using the 1 lb-force calibrated condenser. The final amalgam

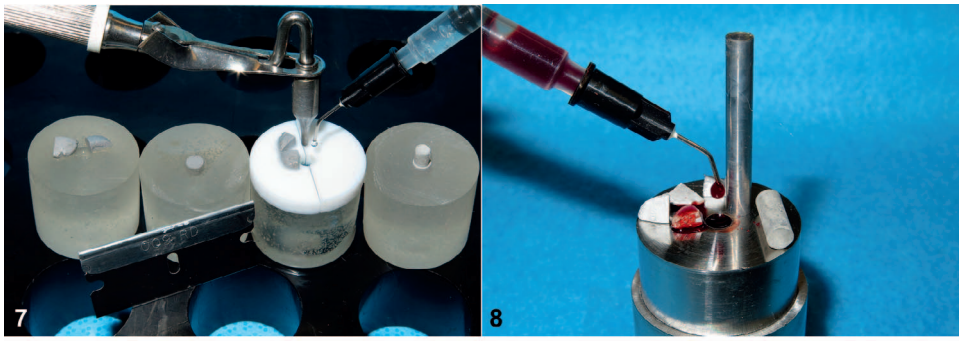


Figure 7. *Series of four resin anchor-wells demonstrating (a) two large quarters of fresh amalgam ready for condensation into the 4 × 4-mm anchor well preloaded with liquid contaminant, (b) condensed, overfilled, and razor-shaved specimen of fresh amalgam, (c) split-ring specimen-forming disc place in place with contaminant loaded first then the two smaller quarters of amalgam condensed while continuously submerged in liquid contaminant, and (d) the final shear-test specimen stub ready for testing at one day or one month.*

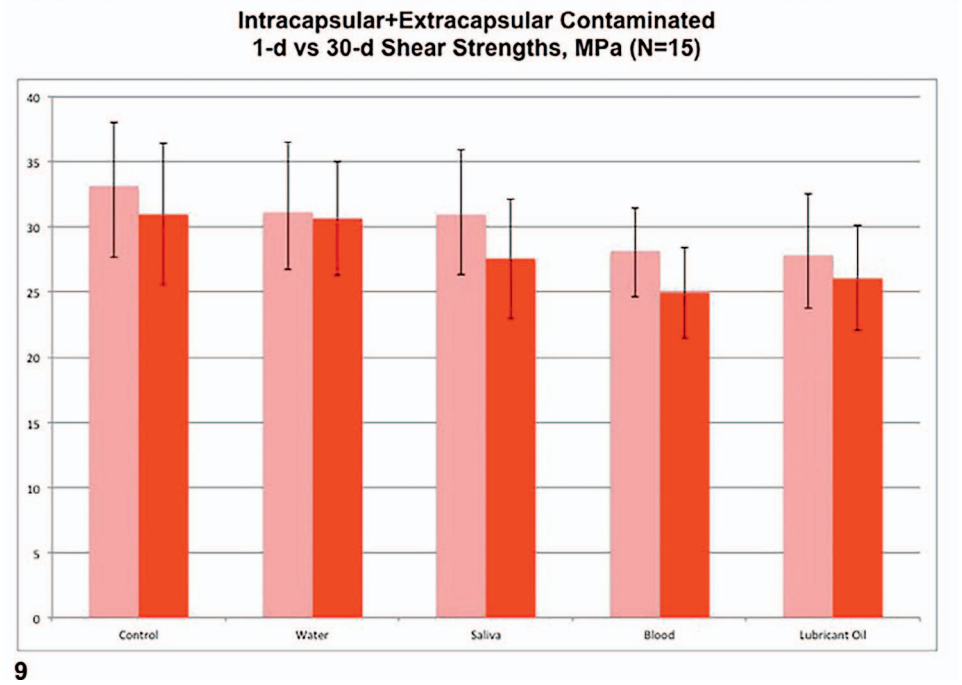


Figure 8. *ADA Specification No. 1 amalgam mold device showing quartered pieces of fresh amalgam ready to be inserted into the central chamber after blood was placed. More blood was added after each increment to keep the amalgam submerged below the blood surface. The piston plunger is standing ready to compress the contaminated amalgam through the central chamber with nearly 40 lb of weight. A final 4 × 9-mm pellet of condensed contaminated amalgam prepared for compression testing is shown lying on the top right of the mold device.*

Figure 9. *Shear strength mean values (MPa) at 1 day (light red) versus 30 days (dark red) following contamination at trituration (intracapsular) + during condensation (extracapsular). N=15.*

increment was similarly contaminant flooded and condensed, with excess amalgam swept away with a burnishing motion using the rounded angular bend of the condenser. The working time for specimens averaged 4.0 minutes ± 30 seconds. Figure 7 demonstrates the overall sequence to form the shear-testing specimens.

Fresh tubes of blood for this investigation were obtained from a hospital medical laboratory with donor identification undisclosed. Saliva was donated primarily by one of the investigators (BC). Water used in this study was deionized. Handpiece oil was Midwest Plus Handpiece Lubricant (part no. 380130, 2 fl oz [59 mL] RJR5, Dentsply Professional, Des Plaines, IL, USA).

All contaminant fluids were replenished with a minimum of five intervals per specimen at an estimated total of at least 0.25 mL to assure that each increment of amalgam was condensed under flooded or submerged conditions.

After a minimum of 2 hours, the 15 resin cylinders were individually pushed out of the Ultradent mold tray to remove their split-ring Delrin specimen-forming molds. Care was exercised not to disturb the standing fresh amalgam specimen stubs or columns. Samples for 24 hour testing were stored at 37°C under 100% humidity, and samples for 30 day testing were stored at 37°C under deionized water. Any inadvertent excess accumulation of amalgam at the base of the columns was carefully removed with a single-edged razor blade prior to testing.

Compressive Strength Specimen Preparation

The central well of the ADA Specification No. 1 amalgam compression-testing mold device was first loaded partway with the contaminant that was appropriate for each sample. Pre-encapsulated amalgam capsules were mixed using a triturator at 10 seconds per capsule according to the manufacturer's instructions. The resulting bolus of amalgam was

Table 3: *Shear Strength Test Results (MPa)*

Group	1-Day (±SD)	p Δ/C	30-Day (±SD)	p Δ/C	p Δ/1-d
Mean intracapsular+extracapsular contaminated amalgam shear strengths compared					
Control (C)	33.08±4.97)	—	30.97±5.41	—	0.032
Water	31.16±5.32	0.061	30.63±4.41	0.076	0.432
Saliva	30.89±5.02	0.539	27.54±4.56	0.202	0.001
Blood	28.12±3.25	0.002	24.92±3.48	0.040	0.038
Lubricant oil	27.82±4.73	0.006	26.06±4.06	0.042	0.198
Mean extracapsular-only contaminated amalgam shear strengths compared					
Control (C)	32.23±4.49	—	27.43±4.62	—	0.047
Water	29.03±5.29	0.164	31.46±3.75	0.015	0.334
Saliva	32.27±4.02	0.998	26.67±3.74	0.760	0.004
Blood	26.60±5.82	0.009	25.35±5.57	0.391	0.496
Lubricant oil	28.17±4.55	0.035	26.68±3.88	0.722	0.551

Sample means in megapascals; N=15. Abbreviations: p Δ/C, p *value of difference from the control mean; p Δ/1-d,* p *value of mean difference between 1-day and 30-day means.*

sectioned into quarters with a single-edged razor blade.

The initial increment of amalgam was directed into the central well or hole of the compression strength mold device and pushed into place through the fluid contaminant using the beaks of the cotton forceps. After assuring inundation with fluid contaminant, the next increment was added, until all four quarters of fresh amalgam were inserted. Layers of fluid contaminant were delivered by syringe to the surface to keep each increment of amalgam continuously covered. After all the amalgam was inside the device, the plunger rod was inserted and placed under compressive weight according to the ADA amalgam Specification No. 1 protocol[14] for compressive strength specimens (Figure 8). The resulting 4 mm-diameter × 9 ± 1 mm-long cylindrical specimens were carefully ejected from the mold device unaltered and stored in deionized water at 37°C until testing. The sample size of six was adopted from previous published compressive strength reports[9,18] and estimated to give 80% statistical power.

Instron Testing of Samples

Shear strength specimens were mounted into a jig (Moran Innovations), which positioned the specimen stubs horizontally to be tested at a 90° angle using an ElectroPuls E3000 Testing Machine (Instron North America, Norwood, MA, USA) with a semicircular notched blade and vertical crosshead speed of 0.5 mm/min. Values were recorded in megapascals.

Compressive strength specimens were tested standing vertically under compression in an ElectroPuls E10000 Testing Machine (Instron North America) with a crosshead speed of 0.5 mm/min and values similarly recorded in megapascals.

The null hypothesis was evaluated using the Kruskal-Wallis/Bonferroni procedure and tested at an α level of 0.05 with IBM SPSS v25 (Cary, NC, USA). The Kruskal-Wallis procedure, the nonparametric correlate of the one-way analysis of variance, was performed due to the smaller sample size and inability to verify assumptions of normality. The Wilcoxon signed ranks test was used to compare the 1-day and 30-day controls.

RESULTS

For intracapsular contamination specimens, the low weight of 0.13 g (<5% of total capsule weight) added by the black vinyl electrical sealing tape including weight of the one drop of contaminant fluid per capsule used in this study was considered not to have a significant effect on the results.

Shear Strengths

Mean shear strength reductions for 1-day samples contaminated both at trituration and during condensation (intracapsular+extracapsular) are shown in Table 3 and Figure 9. There was a consistent, although not statistically significant, reduction of strength by each group by 30 days.

One-day shear strength of the extracapsular saliva-contaminated sample (32.27 MPa) was equivalent to the control (32.23 MPa). This was repeated from a previous run yielding 33.6 MPa, apparently confirming this value.

Table 4: *Fracture Sites Examined Under Magnification*

Fx Sites	C1-E	W1-E	S1-E	B1-E	L1-E	C1-I	W1-I	S1-I	B1-I	L1-I	C30-E	W30-E	S30-E
Interface	1	1	0	5	2	0	0	0	1	0	3	1	2
Mixed	3	2	4	1	1	1	0	2	1	0	1	2	2
Bulk	11	12	11	9	12	14	15	13	13	15	11	14	11

These fracture sites were categorized as follows: interface, >3/4 intact surface; mixed, in between; bulk, <1/4 intact surface. Abbreviations: B, blood contaminated; C, control; E, extracapsular contamination (during condensation); I, intracapsular contamination (at trituration); L, lubricant oil contaminated; S, saliva contaminated; W, water contaminated; 1, 24 hours; 30, 30 days (month).

Thirty-day shear strength p values (p Δ/C) for the intracapsular+extracapsular sample group were water (p=0.076), saliva (p=0.202), blood (p=0.040), and lubricant oil (p=0.042), with the water-, blood-, and lubricant-contaminated groups rejecting the null hypothesis (Table 3).Among the shear strength samples, there was a general tendency for interfacial fractures (across the specific deliberate plane of contamination) in the saliva and blood samples (Table 4). Bulk fractures occurred more often than interfacial fractures, averaging 9 of 15 in saliva and blood extracapsular groups, and for saliva and blood intracapsular groups, the average was 13 of 15. Because this trend was similar throughout all samples, there may have been sufficient weakening from intracapsular contamination saturation at trituration that predisposed specimen fractures through the bulk rather than at the interface.

Any additional effect that intracapsular contamination had on shear strength occurred consistently regardless of contaminant source but not statistically significant (p=0.05, Table 3, Figure 10).

Compressive Strengths

Mean strengths for 1 day compressive samples contaminated both at trituration and during condensation were lower than the 1 day control but consistently increased after 30 days to the following: control, 447.7 ±76.3 MPa; water, 343.6 ±70.1 MPa; saliva, 307.7 ±24.0 MPa; blood, 281.6 ±35.2 MPa; lubricant oil, 227.8 ±16.9 MPa. The blood-contaminated amalgam value in the intracapsular+extracapsular group was diminished, 157.4 ±39.9, at 1 day but recovered significantly after 30 days (Table 5, Figure 11).

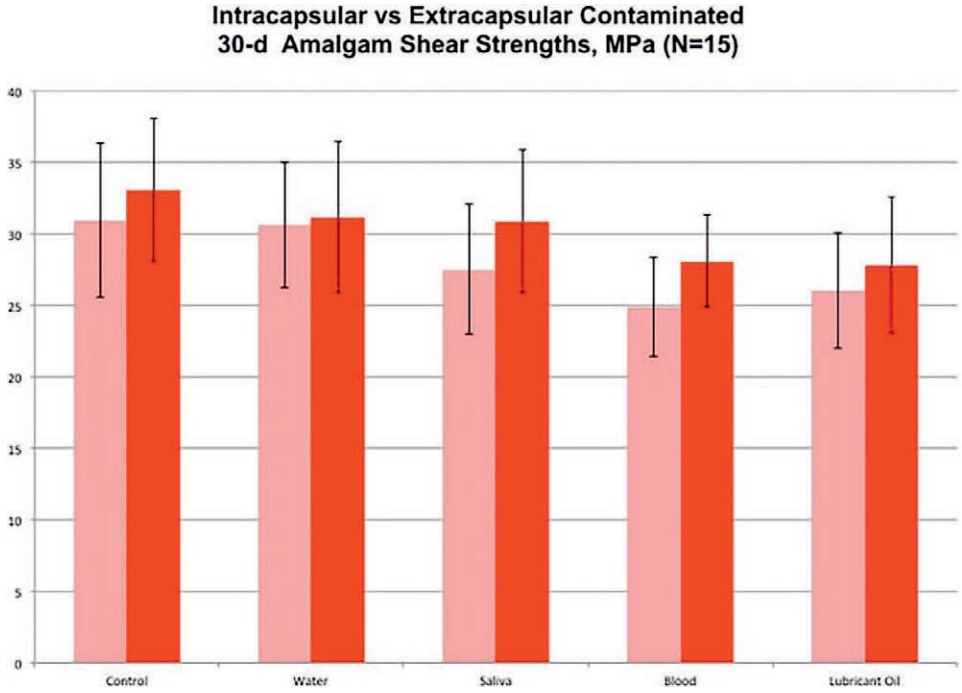


Figure 10. *Shear strength mean values (MPa) at 30 days following contamination at trituration (intracapsular+extracapsular, light red) + during condensation versus during condensation only (extracapsular, dark red). N=15.*

Table 4: *Fracture Sites Examined Under Magnification (ext.)*

Fx Sites	B30-E	L30-E	C30-I	W30-I	S30-I	B30-I	L30-I
Interface	2	0	0	0	0	1	0
Mixed	3	1	0	0	2	1	1
Bulk	10	14	15	15	13	13	14

Compressive strength values below the selected minimum of 241 MPa, corresponding to the ADA-suggested minimum of 35,000 psi,[9] were primarily in the 1 day (blood, 157.4 ±39.9 MPa; lubrication oil, 188.4 ±52.5 MPa) samples. At 30 days, only the lubricant oil (227.8 ±16.9 MPa) fell below this hypothetical level (Table 5, Figures 11 and 12).

Compressive Strength Results Relative to Contaminants

Water-contaminated samples complied with the null hypothesis, whereas other contaminated samples were rejected ($p>0.05$).

After 30 days, only lubricant oil, when permeated throughout the amalgam mass by both trituration and during condensation, had strength at or below the suggested minimum standard of strength (Table 5, Figure 12).

Compressive Strength Results Relative to Shear

In general, all sample mean intracapsular+extracapsular compressive strengths increased between 1 day and 30 days (Table 5, Figure 11). The converse was found for shear strengths with all intracapsular+extracapsular samples diminishing in value but not to a statistically significant extent (Table 3, Figure 9).

Intracapsular Contamination (at Trituration) Results Relative to Extracapsular Contamination (During Condensation)

As a means of introducing contamination, placing a drop inside the amalgam capsule prior to trituration was potentially more thorough than flooding during condensation alone. This was the method chosen by many amalgam investigators (Table 2), and, especially for compressive strengths, its detrimental effect was slightly enhanced beyond extracapsular contamination alone. This effect was dramatized more in the 1 day than in the 30 day amalgam samples. For example, MPa strengths in the saliva contamination group were reduced from 313.2 ± 38.9 to 252.1 ± 31.7 at 1 day and from 326.7 ± 65.9 to 307.7 ± 24.0 at 30 days. In keeping with worst case scenario prospects, long-term conclusions in the present study have been based on intracapsular+extracapsular contamination with 30 day compressive strength results.

DISCUSSION

Dental amalgam has gained the reputation as a relatively safe restorative material to use in situations where isolation against moisture contamination is compromised. Taking it to an extreme, this study attempted to answer what the worst effect on the final strength of amalgam might be, ascertained from overt contamination by total inundation of amalgam during placement. Individual specimens needed to be prepared in a strictly uniform manner

Table 5: *Compressive Strength Test Results (MPa)*

Group	1-Day (±SD)	p Δ/C	30-Day (±SD)	p Δ/C	p Δ/1-d
Mean intracapsular+extracapsular contaminated amalgam compressive strengths compared					
Control	414.3±85.3	—	447.7±76.3	—	0.530
Water	301.2±22.6	0.099	343.6±70.1	0.187	0.028
Saliva	252.1±31.7	0.017	307.7±24.0	0.006	0.084
Blood	157.4±39.9[a]	<0.001	281.6±35.2	0.001	0.182
Lubricant oil	188.4±52.5[a]	<0.001	227.8±16.9[a]	<0.001	0.875
Extracapsular-only contaminated amalgam compressive strengths compared					
Control	430.5±36.1	—	441.3±67.9	—	0.463
Water	368.1±43.1	0.251	402.2±36.4	0.577	0.028
Saliva	313.2±38.8	0.017	326.7±65.9	0.045	0.917
Blood	273.3±41.8	0.008	247.5±103.2	0.026	0.753
Lubricant oil	260.6±29.5	0.002	216.7±23.8[a]	0.002	0.116

Sample means in megapascals; N=6.
[a] Mean values are less than 35,000 psi (241 MPa) ADA-suggested[9] minimum strength.

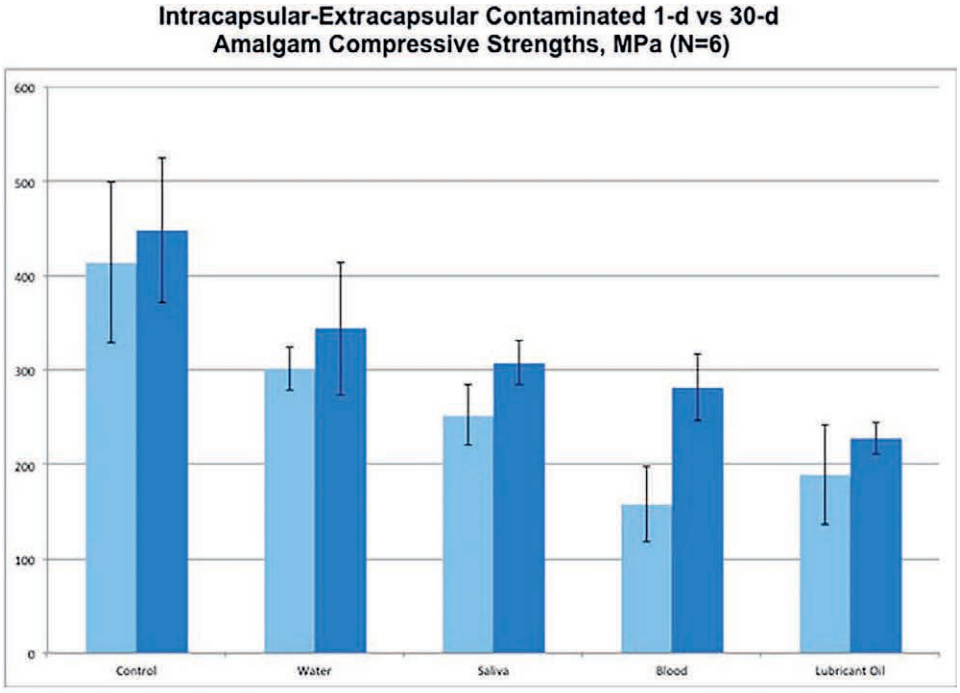


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Figure 11. *Compressive strength mean values (MPa) at 1 day (light blue) versus 30 days (dark blue) following contamination at trituration (intracapsular) + during condensation (extracapsular). N=6.*

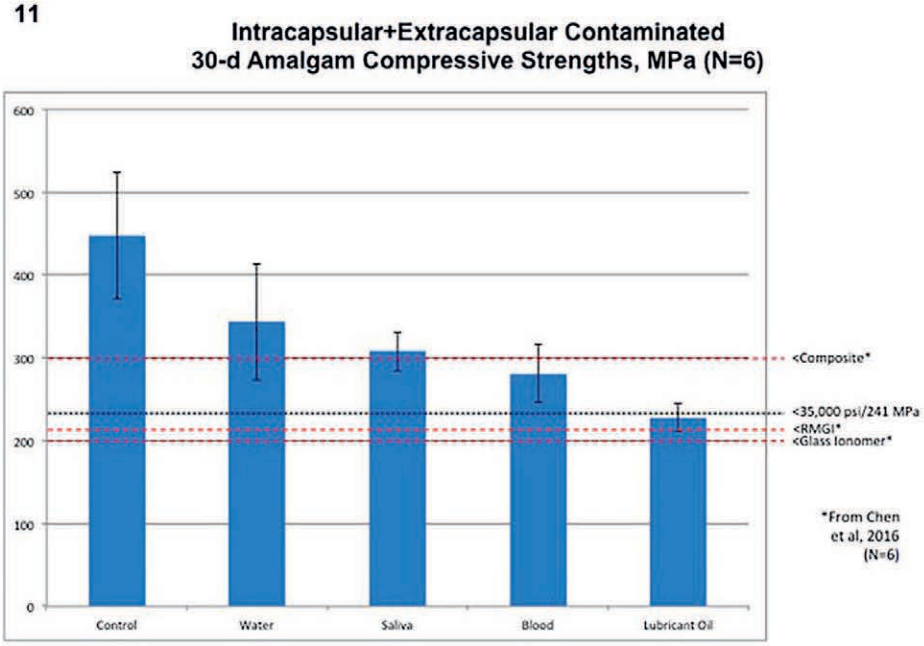

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Figure 12. *Compressive strength mean values (MPa) at 30 days following contamination at trituration (intracapsular) + during condensation (extracapsular). Bar graph display of variously contaminated amalgam strengths, with dashed lines indicating intact uncontaminated strengths of a posterior composite resin material (Quixfil, Dentsply Sirona), typical resin-modified glass ionomer (RMGI; Fuji II, GC America), and conventional glass ionomer (GI; Fuji IX, GC America) compressive strengths from Chen and others.[19] The line at 35,000 psi (241 MPa) indicates the suggested minimum compressive strength recommended historically by the ADA for amalgam.[9] N=6.*

to limit the manipulative variables affecting the compressive and shear strengths.

The protocol required regular and thorough condensation, complete inundation of amalgam during condensation that would not interfere with consistent quality of condensation, and a system of parallel analysis (shear testing) as a side reference to the traditional method of compressive testing.

Of particular interest was the relative reduction in strength resulting from potential chairside contaminants when introduced overwhelmingly during amalgam condensation. It was also of interest to investigate changes induced after 30 days by contamination at the trituration stage where distribution would be total and homogeneous and between aqueous-based and oil-based contaminants.

Contaminants generally recognized as a potential threat to ideal amalgam placement were selected: water, saliva, blood, and oil. Handpiece lubricant oil was included in this study because it is considered a potential source of contamination from handpiece spray. It was useful to learn if such a nonaqueous contaminant might degrade amalgam strength beyond that produced by water-soluble contaminants. Despite thorough contamination by handpiece lubrication oil agents prior to and during amalgam condensation, at 30 days the retained compressive strength was nearly 50%.

Valiant PhD-XT is a non-zinc, high-copper, phase-dispersed alloy admixture. Each Sure-Cap capsule contained 800 mg alloy and 746 mg Hg, with a slower setting time (XT indicates extended time). The working time for specimens in all groups of the present study averaged 4.0 minutes ± 30 seconds, which was within the manufacturer's stated working time of seven minutes.

The condensation force of 1 lb (0.45 kg) with a 1.5 mm-diameter flat nib was determined empirically to be optimal because greater force than this resulted in greater penetration through the amalgam than actual compaction.

The 24 hour compressive strength of Valiant PhD was rated by the manufacturer at approximately 75,000 psi (517 MPa).

What Is the Critical Level of Weakness Relative to Clinical Acceptability?

Compared with other restorative materials such as composite resin or direct gold, amalgam strength is purportedly superior when contaminating conditions cannot be totally controlled. Literature sources, however, were indecisive about what an actual critical threshold of clinical acceptability should be. ADA Specification No. 1 stated only a minimum of 80 MPa tested one hour following ejection from the dental amalgam specimen mold.[14]

ADA Specification No. 1 historically had required a minimum compressive strength of 35,000 psi (241 MPa) at 24 hours.[9] Less strength was conjectured to increase the possibility of fracture under normal biting stress. For durability of the material to resist normally expected clinical biting loads, the degree loss of amalgam strength is not considered as important as maintaining strength above the suggested minimum.[9]

It is not known specifically what the desired minimum strength needs to be for amalgam. When there is adequate bulk, such as in restorations with deeper and wider preparation designs, the inherent alloy strength does not need to be as great.[18] The minimum limit would also vary according to other factors such as stress locations involving central compressive versus edgewise shearing forces.

The period of time when the amalgam is setting is most critical; it has been estimated that 85% of the strength is attained by eight hours after placement.[18] Strength often increases slightly after 24 hours, which has been associated with continued chemical reactions. Phillips suggested that equilibrium of amalgam in the mouth is reached only after an indefinite period of time, if ever.[18]

Compressive Strength Results Relative to Other Dental Materials

The Instron E3000 load cell used for amalgam shear strength testing was inadequate for compressive testing, which instead required use of the Instron E10000.

It is useful to compare these values with the normal compressive strength of other resin-based restorative products that might have substituted for amalgam. Compressive strength values of the control and water-, saliva-, and blood-contaminated groups after 30 days was still greater than a typical resin-modified glass ionomer (RMGI) manufacturer's[19] stated value of 230-274 MPa (for Fuji II LC, GC America, Alsip, IL, USA) and that was "indicated for stress-bearing Class II restorations." Mean contaminated amalgam values were also considerably greater than the 178 MPa for a conventional glass ionomer (GI; Fuji IX, GC America, Alsip, IL, USA) restorative material.[20]

The mean compressive strength of amalgam 30 days after maximum contamination by saliva, which is the chief contaminant under oral conditions, was 326 ± 65.9 MPa. This value was roughly equivalent to the 200 to 340 MPa textbook range for compressive strength of microhybrid composite and 230-290 MPa for microfilled composite.[21]

Comparable to the present study, a laboratory report by Chen and others[22] with a conventional glass ionomer (Fuji IX), a resin-modified glass ionomer (Fuji II LC), and a posterior composite resin (Quixfil, Dentsply Sirona, York, PA, USA) showed compressive strengths of 203, 217, and 300 MPa, respectively. These results[22] are particularly relevant in that the GI, RMGI, and posterior composite samples were incubated underwater for 30 days and tested similar to the present protocol (Figure 12).

The 30 day mean compressive strengths of water- and saliva-contaminated amalgam in the present study exceeded the reported values for uncontaminated posterior composite, RMGI, and GI in their study. Also, the blood- and oil-contaminated amalgam strengths were greater than their RMGI and GI values (Figure 12). It should be noted that in actual practice, contamination with either blood or handpiece oil would be highly improbable during the condensation of amalgam.

The maximally contaminated saliva sample after 30 days still retained a compressive strength above the suggested ADA minimum recommendation for amalgam.[9]

Why Test for Shear Strength in Addition to Compressive Strength?

Compressive force, which assesses the overall bulk of amalgam, is the standard method of evaluating amalgam strength. Amalgam, however, can be subject more to tensile than to compressive failure under certain clinical stress conditions. For this reason, shear strength testing was included.

From a previous study, a method of continuously condensing a column of amalgam[17] was adopted to assure not only complete submersion of the amalgam as it was being condensed but also that the contaminating agent was applied directly at the shear-force interface to avoid question whether the actual testing surface had been contaminated. This was considered by the authors to be more specifically focused than other traditional ISO testing methods for estimation of amalgam shear strength under "worst case scenario" conditions, as Nelson and Mahler[12] had described.

The significance of the present study design was that shear testing occurred perpendicular to the discrete plane of known contamination. Although the amalgam had already been condensed while totally submerged in fluid contaminants in addition to contamination at trituration, specific contamination at that particular interface is considered the most crucial. If the inherent interface joint strength was great enough not to cleave at that plane, the amalgam would fracture more angularly into the bulk. In this way, shearing action became a direct test of weakness induced by the contaminant at what might be considered the most vulnerable location.

However, seldom did the fracture occur at the interface. This may indicate that the patterned condensation with the 1.5 mm-diameter 1 lb-force condenser on the plane of specifically known contamination allowed the added increments of amalgam to join effectively despite interference from the contaminants.

Amalgam shear strength after placement was degraded during the following 30 days under water at 37°C.

Compressive forces consist of a component of tensile stress in expansion.[23,24] The compressive test results in the present study were more definitive than the shear strength results. However, in actual clinical cases, it is less frequent that amalgam fails by direct perpendicular compressive force alone. Amalgam failures are generally noted from tensile or shear action in relatively unsupported areas such as in isthmus fractures.[18] In this regard, shear-testing data are considered relevant.

Possible Mechanism of Action for Amalgam Contamination Resistance

Mercury is a metal that uniquely exists in a liquid state at ambient temperature. This molten state allows small particles of other metals to combine and solidify to form an amalgam. The ability of amalgam to flow together and join with itself in a coherent mass owes to the capacity of the mercury to unite with other increments of liquid mercury in the mixture around and through the contaminating fluid. As mercury components coalesce under condensation forces, non-mercuric liquids are apparently expressed outside the amalgam mass. It is postulated that proteinaceous elements of blood and saliva may cling to particles of amalgam alloy and are not as readily expelled. The compressive specimens that had been contaminated with saliva and blood were approximately 0.5-1.0 mm longer than control or water- or oil-contaminated specimens. This was assumed to be due to the residual incorporation of mucin and formed blood elements within the mass, which were unable to be fully squeezed out under condensation pressure.

There may be properties of handpiece oil that likewise have an affinity for solid metallic particles. It is speculated that this factor could cause inhibition of significant chemical actions that ordinarily would have occurred and prevented strengthening of the amalgam during the 30-day incubation storage period.

The remaining amalgam strength of 35,000 psi at 30 days as a suggested minimum value, based on a past ADA recommendation for minimum acceptability,[9] may still be considered reasonably functional.

Mean compressive values for the water-, saliva- and blood-contaminated samples, in the group

including both intracapsular and extracapsular contamination, indicated amalgam strength after 30 days to be at least comparable to published values for intact RMGI. Mean values individually for water-, saliva-, blood-, and oil-contaminated samples after 30 days were superior to the published value[17] of intact uncontaminated GI cement (Figure 12 shows 30-day compressive strengths).

Power and sample size analysis conducted from a prior pilot study anticipated a larger effect size. For data in which the null hypothesis was retained, no post hoc power analysis was conducted.

It is recognized that final conclusions about amalgam strength cannot be determined solely by compressive and shear testing. However, although salivary contamination is recognized as the main challenge clinically, thoroughly condensed amalgam may be capable of providing a functional restoration, even if overwhelmingly challenged by water, saliva, blood, or lubricating oil contaminants.

To conduct studies on actual patients with the amalgam placed totally under these contaminating factors would likely be considered unethical. The closest viable alternative is laboratory testing, from which results the clinician should be able to prognosticate a certain range of outcomes and judge their practicality.

Testing of alternative resin-based restorative products for comparison with an amalgam alloy was desired but not considered feasible due to inherent limitations of the materials. Otherwise, future studies might include testing whether direct gold, glass ionomer, or composite resins can sustain similar contamination without undue debilitation of strength and integrity.

It would be of interest to study whether use of an automatic mechanical condensing device instead of hand condensation would provide greater amalgam strength results under similar conditions. Also, amalgam tensile type testing could be accomplished using both a lower and an upper split-ring mold design to produce specimens for analysis in a three-point stress test, directed across planes of deliberately induced contamination.

CONCLUSIONS

- Under conditions of this study, contaminants reduce amalgam 30-day compressive strength in increasing order: water, saliva, blood, and handpiece lubricant oil.
- Of the water, saliva, blood, and handpiece lubricant oil contaminants tested, only water complies with the null hypothesis having no significant effect on amalgam compressive strength ($p>0.05$).
- Despite thorough contamination of amalgam by water, saliva, blood, or handpiece lubricant oil contamination agents, prior to and during condensation, the greatest mean reduction in compressive strength at 30 days is 44% by blood and 51% by the oil.
- Amalgam 30-day mean shear strength is significantly altered by contamination with water, blood, or handpiece lubricant oil ($p<0.05$).
- With total contamination at placement, mean amalgam compressive strength after 30 days is comparable with uncontaminated composite resin and resin-modified glass ionomer and substantially greater than uncontaminated glass ionomer under similar reported laboratory conditions.

Acknowledgements

This study was supported by the Loma Linda University School of Dentistry Student Research Program and a seed grant. The authors are indebted to Dr. Serkan Inceoglu at the Orthopedic Research Laboratory for personal collaboration and use of the Instron E10000 for the compressive strength samples. There is special gratitude to Drs. Henry Lukic and Max Gruber at the American Dental Association for loaning use of their Specification No. 1 amalgam mold for compressive strength testing, and to Dr. Udochukwu Oyoyo for performing the statistical analyses.

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.

(Accepted 7 August 2018)

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Delayed Photoactivation of Dual-cure Composites: Effect on Cuspal Flexure, Depth-of-cure, and Mechanical Properties

KO Hughes • KJ Powell • AE Hill • D Tantbirojn • A Versluis

Clinical Relevance

Delaying two minutes before light-curing after placing dual-cure composites can take advantage of shrinkage stress reduction of the slower self-cure component before the more rapid photoactivation without impacting curing depth or mechanical properties.

SUMMARY

Objectives: This study tested whether delayed photoactivation could reduce shrinkage stresses in dual-cure composites and how it affected the depth-of-cure and mechanical properties.

Methods and Materials: Two dual-cure composites (ACTIVA and Bulk EZ) were subjected to two polymerization protocols: photoactivation at 45 seconds (immediate) or 165 seconds (2 minutes delayed) after extrusion. Typodont premolars with standardized preparations were restored with the composites, and cuspal flexure caused by polymerization shrinkage was determined with three-dimensional scanning of the external tooth surfaces before restoration (baseline) and at 10 minutes and one hour after photoactivation. Bond integrity (intact interface) was verified with dye penetration. Depth-of-cure was determined by measuring Vickers hardness through the depth at 1-mm increments. Elastic modulus and maximum stress were determined by four-point bending tests (n=10). Results were analyzed with two- or three-way analysis of variance and pairwise comparisons (Bonferroni; α=0.05).

Results: Delayed photoactivation significantly reduced cuspal flexure for both composites at 10 minutes and one hour ($p \leq 0.003$). Interface was >99% intact in every group. Depth-of-cure,

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DOI: http://doi.org/10.2341/18-140-L

elastic modulus, and flexural strength were not significantly different between the immediate and delayed photoactivation ($p>0.05$). The hardness of ACTIVA reduced significantly with depth ($p<0.001$), whereas the hardness of Bulk EZ was constant throughout the depth ($p=0.942$).

Conclusions: Delayed photoactivation of dual-cure restorative composites can reduce shrinkage stresses without negatively affecting the degree-of-cure or mechanical properties (elastic modulus and flexural strength).

INTRODUCTION

Composite resin materials shrink when they polymerize, which can result in marginal failure, postoperative sensitivity, microleakage, secondary caries, and even tooth fracture.[1] In his landmark article that started the revolution of dental composites, Bowen pointed out that polymerization shrinkage would exert a force that could pull the material away from the cavity walls only after it reached a critical stiffness.[2] Before the composite reaches structural stiffness, contraction stress can still be relieved by viscous flow. The stress relieving flow can be extended by reducing the rate of polymerization, resulting in a decrease in shrinkage stress.[3,4] This concept has been clinically applied by moderating the light intensity during photoactivation, as in the soft-start and pulse-delay photoactivation techniques.[5-8]

Slow polymerization has been a characteristic of self-cure composites because their polymerization process spans several minutes compared to the 20-40 seconds of most photoactivated composites.[4] Reportedly, the cure speed of photoactivation could be up to 322 times faster than with self-curing.[9] Recently dual-cure restorative composites were reintroduced that combine both curing mechanisms.[10-12] Dual-cure composites can presumably overcome curing depth limitations and thus become a candidate for bulk-fill procedures. Dual-cure composites may also allow further reduction in shrinkage stresses if the benefits of self-cure (low polymerization rate) and photoactivation (cure on demand) can be exploited using a delayed photoactivation technique. In this technique, photoactivation is delayed for a few minutes after the start of the mixing procedure to take advantage of the slow polymerization system of the self-cure component.[13,14] This time delay has been shown to improve bonding of resin cements[15,16] and reduction of shrinkage stress in core build-up materials and resin cements.[13,14,17] However, reduction in shrinkage stress has not been tested yet for dual-cure restorative composites. Moreover, questions remain if delayed photoactivation can have negative effects on mechanical properties. The literature is not consistent on this concern.[13,17,18]

The objective of this study was twofold: (1) determine the influence of delayed photoactivation of dual-cure restorative composites on polymerization shrinkage stresses and (2) evaluate the effect of delayed photoactivation on depth-of-cure and mechanical properties (elastic modulus and flexural strength). The hypotheses were that delayed photoactivation of dual-cure composites would reduce residual shrinkage stress and their mechanical properties.

METHODS AND MATERIALS

Restorative Composites and Curing Regimens

Two dual-cure composite materials—ACTIVA BioActive Restorative (Pulpdent Corporation, Watertown, MA, USA) and Bulk EZ (Danville Materials, Carlsbad, CA, USA)—were subjected to two curing regimens: 45 (immediate) or 165 seconds (with a 2-minute delay) after extrusion (mixing) of the composites. Material information is listed in Table 1.

Cuspal Flexure

Shrinkage stress conditions were assessed by measuring cuspal flexure of restored teeth. Typodont teeth were used for the cuspal flexure experiments. A previous study showed that cuspal flexure experiments with typodont teeth compared well with the cuspal flexure of natural teeth, replicating their stress distributions while standardizing the test conditions by precluding natural tooth variations.[19] Forty typodont maxillary second premolars (Nissin–Kilgore, Nissin Dental Products, Inc, Kyoto, Japan) with prefabricated 3-mm-wide × 3-mm-deep mesio-occlusal-distal (MOD) preparations were securely mounted in stainless steel rings (Figure 1A). All teeth were sandblasted with a MicroEtcher II Intraoral Sandblaster (Danville Materials, San Ramon, CA, USA) and Cojet Sand (3M Deutschland GmbH, Neuss, Germany) to provide adhesion. Scotchbond Universal adhesive (3M Deutschland GmbH) was applied to the cavity and rubbed for 20 seconds. A gentle stream of air was applied for about five seconds to evaporate the solvent. The adhesive was then light-cured for 10 seconds and followed by placement and 20-second light-cure of the dual-cured composites. The composites were either immediately light-cured (45 seconds after extrusion) or delayed light-cured (165 seconds after extrusion). All light

Table 1: *Material Information*

Description	Material Information	Manufacturer	Composition
Bioactive dual-cure composite	ACTIVA BioActive-Restorative Shade A2 Lot 170126, 170326	Pulpdent Corporation	Patented, ionic-resin (Embrace resin), shock-absorbing resin component 56% by weight fillers (21.6% reactive ionomer glass fillers)
Dual-cure bulk fill composite	Bulk EZ Shade A2 Lot 47800, 123HU	Danville Materials	Patent-pending self-cure IntelliTek Technology
Typodont teeth Upper right second premolar	A25SAN-UR59M Lot 704808	Nissin Dental Product Inc	Bisphenol A diglycidyl ether epoxy resin 36-63 wt% Butyl glycidyl ether 4-8 wt% Inorganic Substance 29-52 wt% Colorant 0-8 wt%
Sandblast	3M ESPE CoJet Sand Lot 610969	3M Deutschland GmbH	Silicatized sand, particle size 30 μm
Bonding agent	3M ESPE Scotchbond Universal Lot 619547	3M Deutschland GmbH	Bis-GMA, HEMA, dimethacrylates, ethanol, water, methacrylate functional copolymer of polyacrylic and polyitaconic acids, and photoinitiator 5 nm silane-treated spherical silica particles, 10% by weight

Sources: Product's Safety Data Sheet, Product Profiles, Product website, Product Technical Manual. Abbreviations: bis-EMA(6), bisphenol A polyethylene glycol diether dimethacrylate; Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; PEGDMA, polyethylene glycol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, diurethane dimethacrylate.

curing was carried out with an LED light-curing unit (Kerr, Orange, CA, USA) in standard curing mode with 1517 ± 10 mW/cm^2 output according to the MARC Patient Simulator (BlueLight Analytics, Halifax, Nova Scotia, Canada). Sample size was 10 per group.

The samples were scanned (COMET xS Optical Scanner, Steinbichler Optotechnik GmbH, Neubeuern, Germany) before composite placement (baseline), at 10 minutes and one hour after restoration. Tooth surfaces from the post-restoration scans were aligned with the baseline scan by precisely aligning

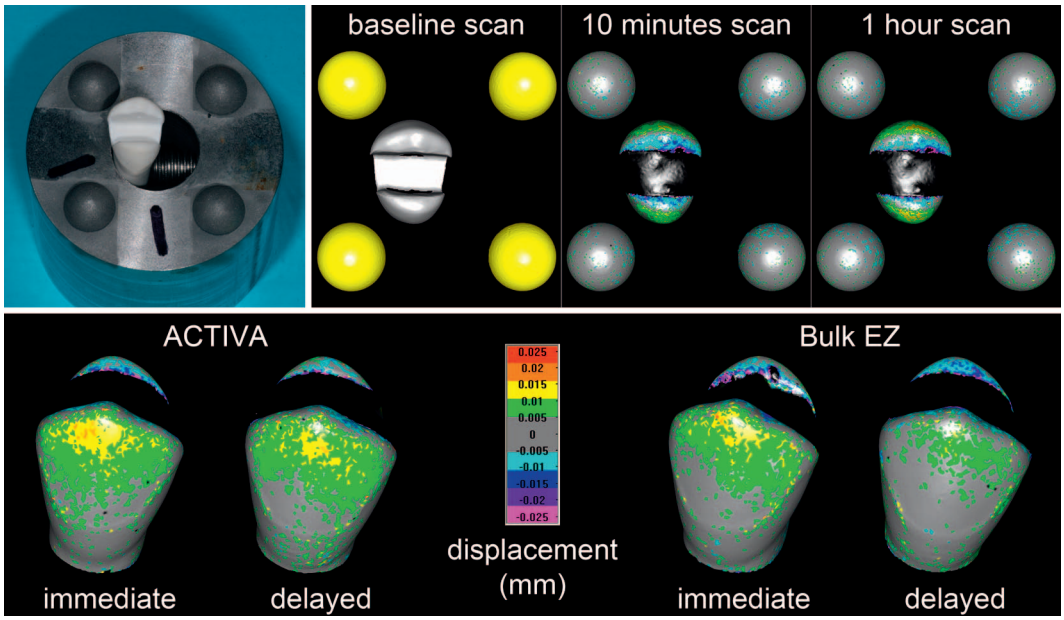

Figure 1. *(A) Typodont tooth with MOD preparation fixed in a stainless steel mold with embedded reference spheres. (B) The 10-minute and one-hour restoration scans were precisely aligned with the preparation (baseline) scan using the reference spheres (yellow). Cuspal flexure was determined by calculating the difference between the restored tooth surfaces and the baseline. (C) Visualization of cuspal flexure of the four experimental groups using a color scale.*

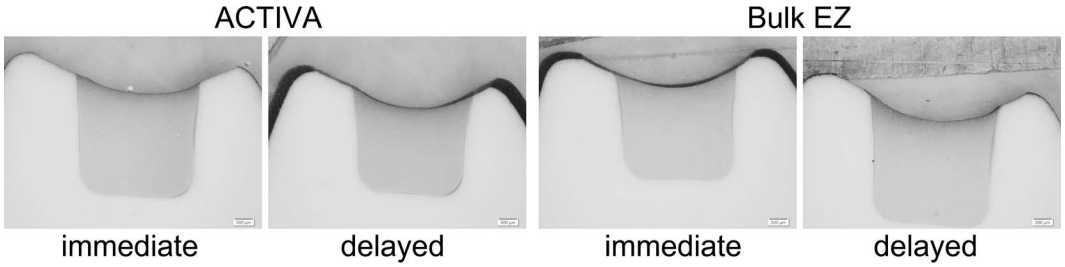

Figure 2. *Samples from dye penetration experiment showing excellent bond integrity at the occlusal interfaces in every group.*

reference spheres on the stainless steel rings using Cumulus software (copyright regents of the University of Minnesota) (Figure 1B). Cuspal flexure was calculated from the difference between the baseline and restoration scans for the buccal and lingual surfaces, calculated using custom developed software (CuspFlex).[19] The effect of composite, regimen, and time was statistically analyzed using three-way analysis of variance (ANOVA; α=0.05).

Bond Integrity

Intact bonding (tooth restoration interface integrity) was verified because it is important for the validity of the cuspal flexure results. Once all scans were completed, the restorations were finished using a 12-blade carbide bur to remove excess composite (flash covering occlusal margins) on the occlusal surface. The teeth were placed in 0.5 wt% basic fuchsin dye for 16 hours. The teeth were then sectioned buccolingually into 1-mm slides (Figure 2), and dye penetration along the occlusal interfaces was measured by two independent evaluators using a stereomicroscope with a CCD camera (SZX16 and UC30, Olympus, Tokyo, Japan). Percentage of intact bond was determined by dividing the length of the intact interface by the wall length (cavity depth). The results of both evaluators were averaged, or a consensus value was reached if the difference between the evaluators was more than 10%. The bond integrity results were statistically analyzed using two-way ANOVA (α=0.05).

Depth-of-cure

Microhardness was used to assess the depth-of-cure. The composites were extruded in the rectangular slot (1.5 mm wide $\times$ 2 mm high $\times$ 8 mm long) of a plaster mold. The slot was covered with an orange glass plate (Bullseye Glass Company, Portland, OR, USA), while the end of the slot was covered with a thin clear glass coverslip (0.15 mm thick). The curing light tip was placed against the clear glass cover slip at the end of the covered slot to allow photoactivation according to the two curing regimens. This configuration ensures that the curing light irradiates the composite from one direction.[20] Sample size was 10 per group.

After one hour, the glass covers were removed, and Vickers hardness was measured (QV-1000 Micro Hardness Tester, Qualitest USA LC, Fort Lauderdale, FL, USA) on the composite surfaces. This provided an indication of the degree of cure at various depths, from 0 to 6 mm. The hardness values were plotted along the depth to create depth-of-cure profiles. Statistical differences between curing regimens and depths were analyzed using two-way ANOVA and pairwise comparisons (Bonferroni; α=0.05).

Elastic Modulus and Flexural Strength

Elastic modulus and flexural strength were measured to assess mechanical properties. Elastic modulus is the stiffness of the material, which determines its resistance to deformation and determines the stress values and distribution. Flexural strength represents the failure strength of the material.

The composites were extruded in the rectangular slot (2 mm deep $\times$ 2.5 mm wide $\times$ 25 mm long) of a vinyl polysiloxane mold. The filled mold was placed between two clear glass slides. Each composite sample was then polymerized, with photoactivation starting at 45 or 165 seconds after extrusion for the immediate or delayed photoactivation regimens, respectively. To ensure that the whole beam was light-cured, the tip of the curing light was moved along its length; both sides of the beam were light-cured (each side for a total of 80 seconds). Ten minutes after curing, the samples were removed from the mold and finished with 600-grit silicon carbide paper, after which the depth and width of each beam were measured with a digital caliper.

Beams were subjected to a four-point bending test one hour after cure in a universal testing machine (Instron 5567, Instron Corp, Norwood, MA, USA) at

Table 2: *Cuspal Flexure, Bond Integrity, Elastic Modulus, and Flexural Strength (Mean ± Standard Deviation)*

Parameters	ACTIVA		Bulk EZ	
	Immediate	Delayed	Immediate	Delayed
Cuspal flexure (μm)				
10 minutes	9.4 ± 3.1	8.1 ± 3.0	7.8 ± 2.6	5.5 ± 3.1
One hour	12.4 ± 3.1	10.9 ± 2.5	10.4 ± 2.5	7.7 ± 2.7
Bond integrity (%)	100	100	99.66 ± 0.72	99.96 ± 0.13
Elastic modulus (GPa)	5.1 ± 1.3	6.3 ± 2.8	14.9 ± 2.4	14.5 ± 2.4
Flexural strength (MPa)	18.1 ± 2.0	19.4 ± 2.3	18.6 ± 1.6	19.5 ± 1.2

0.5-mm/min cross-head speed. Support span was 20 mm, with 10 mm between the two loading points. Displacement of the center of the beam during bending was measured using a deflectometer (Model 3540-004M-ST, Epsilon Technology Corp, Jackson, WY, USA). Each sample was loading until fracture. Load and displacement were recorded with Bluehill 2 software (Version 2.6, Instron Corp). Sample size was 10 per group.

The elastic modulus was calculated using $(11FL^3)/(64WH^3d)$, where F is the applied load, L is the distance between the two lower supports, W is the width of the sample, H is the height of the sample, and d is the displacement measured with the deflectometer. Flexural strength was calculated using $(3FL)/(4WH^2)$. The effect of composite and regimen was statistically analyzed using two-way ANOVA (α=0.05).

RESULTS

The tooth cusps flexed inward during polymerization (Figure 1C). Cuspal flexure values of the two composites with immediate or delayed photoactivation measured at 10 minutes and one hour after restoration are shown in Table 2. The table shows that delayed photoactivation reduced cuspal flexure values, whereas cuspal flexure increased further between the 10-minute and one-hour measurement points. The effects of composite (ACTIVA versus Bulk EZ), curing regimen (immediate versus delayed photoactivation), and time (10 minutes versus 1 hour after polymerization) were all significant ($p \leq 0.003$).

The bucco-lingual sections of restored teeth after dye immersion showed hardly any dye leakage (Figure 2). Bond integrity (% intact bond) was higher than 99% in all groups (Table 2). No statistical differences were found for bond integrity between materials (p=0.111) or curing regimens (p=0.206).

Hardness gradually decreased with depth in the ACTIVA composite, whereas hardness remained constant through the depth for the Bulk EZ composite, as shown in the hardness profiles in Figure 3. The statistical analysis confirmed that there was no significant difference in mean hardness values between immediate or delayed photoactivation for either composite (p=0.605 for ACTIVA, p=0.425 for Bulk EZ) and that there was no significant change in hardness throughout the 6-mm depth for Bulk EZ regardless of the curing regimen (p=0.942). For ACTIVA, hardness values decreased significantly until they bottomed out at about 4 mm depth (p<0.001).

The elastic modulus and flexural strength values of the two composites after immediate or delayed photoactivation are also shown in Table 2. The

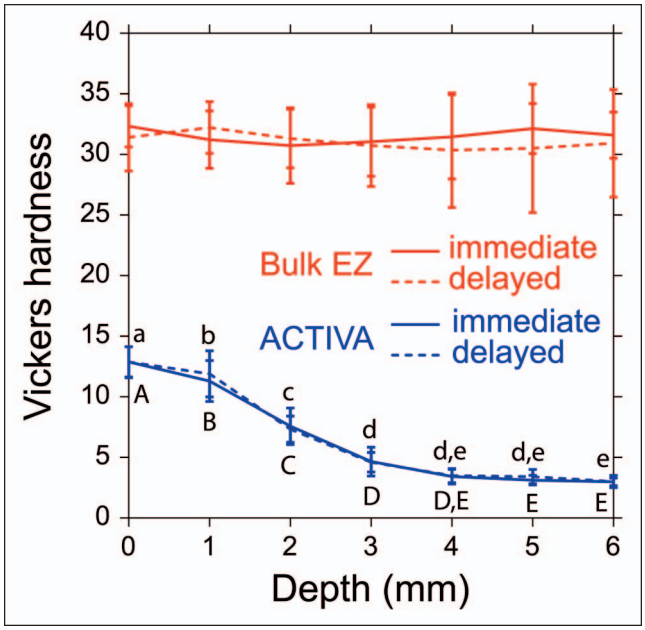

Figure 3. *Depth-of-cure of ACTIVA and Bulk EZ composites with immediate or delayed photoactivation. There was no significant difference in hardness between immediate or delayed photoactivation. There was no significant change in hardness through the depth for Bulk EZ. For ACTIVA, same letters denote hardness values that are not significantly different (two-way ANOVA with pairwise comparison, α=0.05; lowercase letters for immediate, uppercase letters for delayed).*

elastic modulus of ACTIVA was significantly lower than Bulk EZ ($p<0.001$), whereas their flexural strengths were not significantly different ($p=0.597$). Immediate or delayed photoactivation had no significant effect on the elastic modulus ($p=0.509$) or flexural strength ($p=0.067$).

DISCUSSION

The first hypothesis that delayed photoactivation of dual-cure composite restorations reduces shrinkage stress was accepted because the delayed photoactivation regimen reduced cuspal flexure. Before further discussing this finding, three important observations about the testing method need to be made. Shrinkage stress was not measured directly (because stress cannot be measured directly), but cuspal flexure was used as an indication of stresses within the restored teeth.[21] Second, typodont teeth with standardized preparation were used instead of extracted teeth to avoid natural variation in size, shape, and properties while still maintaining clinically relevant stress distributions.[19] This decreased standard deviation and thus increased the resolution of the experiments, an important aspect considering how small the associated cuspal deformation is. Third, cuspal flexure values are only useful as stress analogies if the restorations are bonded. The required bond condition was confirmed with the close to 100% bond integrity. Note that the strength of the bond was irrelevant as long as bond integrity was maintained.

The reduction in shrinkage stress when photoactivation of dual-cure restorative composites was delayed is in agreement with other reports for dual-cure core build-up composites and dual-cure resin cements tested with delayed photoactivation.[13,14,17] As discussed in the Introduction, the reduction in shrinkage stress can be attributed to a reduction in rate of polymerization when the photoactivation component is delayed. A slower polymerization rate is thought to increase pre-gel flow that relieves the accumulation of shrinkage stresses.[5,22] Many studies have confirmed reduction in shrinkage stress in photoactivated composites if photoactivation regimens were altered to slow down the polymerization process,[5,7,8,23] although there are also suggestions that the lower shrinkage stresses might be the result of compromised structural integrity.[24]

Therefore, for our second hypothesis, we tested the possibility that delayed photoactivation compromised structural properties of the dual-cure composites. In the literature there have been various outcomes for dual-cure materials. Some reported that dual-cured resin cements had higher cure with immediate photoactivation,[18] whereas others argued that immediate light exposure could cause formation of polymer chains that interfere with the polymerizing process of the self-cure component by entrapping polymerization promoters.[25] Another study reported that elastic modulus and hardness of dual-cure resin cements were unaffected by a three- to five-minute delayed photoactivation.[17] Our study found no significant differences in hardness profile, elastic modulus, or flexural strength between the immediately or delayed photoactivated dual-cure composites. The second hypothesis was therefore rejected as no evidence was found to suspect that delayed curing had altered or compromised structural integrity of the cured materials. Nevertheless, although our results do not show evidence of compromised integrity, it is still not inconceivable that slight changes in polymer structure were introduced, as others have reported in photoactivated resins.[26] The changes in polymer structure that were reported in the photoactivated resins did not directly affect mechanical properties, but they made the resins more susceptible to ethanol softening. Thus far, such changes appear mostly academic, as they have not been shown to affect clinical performance, which most likely could only show up as long-term effects. Future studies should test long-term mechanical performance of dual-cure composites using artificial aging, as well as leaching of unpolymerized monomers.

The original reason for considering delayed photoactivation was reduction of residual shrinkage stress. Although the negative effect of polymerization shrinkage stress on restoration longevity may be difficult to separate from other long-term challenges faced in the oral environment, shrinkage stress should still be regarded as an unfavorable side effect of resin composites, especially during the first weeks after placement when hygroscopic expansion and stress relaxation have not yet moderated its effects.[27] The ability to reduce shrinkage stress remains therefore a highly desirable option for dental practitioners. However, to adopt delayed photoactivation as a clinical technique, it should also be practical. Clinicians may wonder if it is worth waiting for two minutes before photoactivating. We believe that there are situations where shrinkage stress can be critical, such as in the gingival area of proximal restorations. Maybe two minutes waiting with the bulk-filling technique takes less time than placing two layers of incremental filling. Clinicians

can also limit the time impact by multitasking: for example, using a dual-cure composite to fill the proximal cavity and then filling and shaping the occlusal cavity using regular composite before photoactivating them at the same time. It should also be noted that both manufacturers recommend waiting before photoactivation; for Bulk EZ at least 60 seconds until the material solidifies,[28] and for ACTIVA 20-30 seconds to mitigate polymerization stress and exothermic reaction.[29]

Few dual-cure dental composites are currently commercially available. The advertised advantage of dual-cure composites is their unlimited curing depth, allowing bulk-filling and application in areas where access to light curing is limited. One of the two dual-cure composites in this study (ACTIVA) did not show an unlimited curing depth (Figure 3). Such depth-dependent behavior was also reported in some dual-cure resin cements.[20,30] Bulk EZ had constant hardness throughout the depth, suggesting a more efficient self-curing component. Compositions of both dual-cure composites tested are proprietary and can therefore not be used to explain the outcomes. Notable is that Bulk EZ has a patent pending for IntelliTek Technology for its self-cure component, said to control its shrinkage.[31] Furthermore, in addition to photoactivation and self-curing chemistry, ACTIVA also has a self-cure glass ionomer reaction. In terms of mechanical properties, Bulk EZ had higher hardness and elastic modulus than ACTIVA, whereas the flexural strengths of both dual-cured composites were similar. The low elastic modulus of ACTIVA may in part be due to a patented rubberized-resin component, which the manufacturer claims resists wear, fracture, and chipping.[32] The hardness values of ACTIVA were relatively low and were in the same range as sealant materials and lower than a filled sealant.[33] ACTIVA has been designed to be bioactive and capable of releasing calcium, phosphate, and fluoride to stimulate mineral apatite crystal formation at the material-tooth interface.[29]

Dual-cure restorative composites have not reached the mainstream market. Our own observation is that the major dental manufacturers do not currently have dual-cure restorative composites in their product line. Perhaps the most popular product at the moment is ACTIVA, which may be for its bioactive trait rather than its dual cure.[34] Supplementary finding in this study regarding the depth-of-cure shows that Bulk EZ composite has a more effective self-curing component than ACTIVA and may thus be more reliable as a bulk filling material. For both dual-cure restorative composites, clinicians should be aware that delayed photoactivation is a practical option—although it increases chair time—to reduce shrinkage stress without affecting the depth-of-cure and mechanical properties.

CONCLUSION

Delayed photoactivation of two dual-cure composites reduced shrinkage stresses without negatively impacting depth-of-cure and mechanical properties.

Acknowledgements

This study was supported by the University of Tennessee College of Dentistry Alumni Endowment Fund and the Tennessee Dental Association Foundation. We thank Dr R. DeLong, University of Minnesota, for the Cumulus software.

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.

(Accepted 25 July 2018)

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Volume 44 / Number 2
March/April 2019

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