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Clinical Technique/Case Report

Crown Reconstruction of Erosive Wear Using High-viscosity Glass Ionomer Cement: A Case Report

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D Rios • E Bresciani • AFS Borges • MF de Lima Navarro

Clinical Relevance

High-viscosity glass ionomer cement seems to be an excellent alternative to restore erosive tooth wear lesions, restoring function and aesthetics, in a minimally invasive way.

SUMMARY

The aim of this clinical report is to present a possible alternative treatment, with 24-month follow-up, for restoring tooth loss due to extensive erosive tooth wear. A 21-year-old male patient, complaining of intense sensitivity in the maxillary posterior teeth, and presenting severe wear on maxillary premolar and molar teeth due to gastroesophageal reflux, sought care in the university clinics. The planned treatment was to refer for medical treatment and perform restorations with the high-viscosity glass ionomer cement Equia Forte (GC Corporation, Tokyo, Japan), aiming to restore the dental anatomy and to consequently decrease the pain symptomatology. A silicone guide, obtained from a diagnostic waxing, was used during the restorative approach considering the patient's occlusion. After all the clinical steps of the restorative technique, an occlusal adjustment of restorations was performed. During monthly recalls up to 24 months, the treatment was stable and in service. In addition, the patient reported no pain and improved chewing, leading to a better quality of life.

INTRODUCTION

Studies have shown not only a high prevalence (about 30%) of erosive tooth wear in teenagers[1] but also a

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moderate incidence of this condition in permanent and primary teeth.[2,3] A high progression rate of 25%,[2] in addition to the negative impact of erosive wear on quality of life, especially if associated with tooth hypersensitivity,[4] raises concern.

This condition is a chemical-mechanical multifactorial process not associated with bacterial activity and involving progressive loss of structure when not adequately managed.[5,6] Clinically, the most common signs are concavities in the cusp area and flattened structures with very-well polished surfaces. The reported signs could be generalized or asymmetrically located, depending on the etiology. Despite the difficult task of diagnosing the initial erosion lesions, identifying the etiology of those lesions is essential to prevent the occurrence or progression of the condition.[7] As a multifactorial condition, understanding the patient history combined with the clinical assessment is essential for appropriate management.[6] It is not easy to differentiate lesions originating from erosion, attrition, abrasion, or abfraction, especially because they can present concurantly.[6] However, in some cases, it is possible to identify the predominant causal factor due to the clinical characteristics of the lesion.[6] Abrasion and abfraction lesions can be distinguished from erosive lesions by taking into consideration related factors such as being wedge-shaped with sharp edges. Anyway, there is a need for training dentists in the early detection and monitoring of this process as part of a modern preventive strategy.[6]

Focusing on the erosive component of erosive tooth wear, its etiology is related to intrinsic and extrinsic factors. Identifying the etiology is necessary to manage the causal factors.[6] Information on diet, occupation, recreational activities, past dental procedures, along with the intraoral, head and neck examination aids in understanding the main etiological factors.[6,8] When the etiology is intrinsic in nature, usually from reflux or eating disorders such as bulimia, the erosive lesions initially occur on the palatal surfaces of the maxillary anterior teeth, and subsequently on the lingual and occlusal surfaces of the mandibular teeth. Two controlled trials observed that 60% to 80% of tooth wear is related to gastroesophageal reflux disease (intrinsic factor).[9,10] There is, however, a lack of knowledge by health professionals regarding the association of these dental lesions with the reflux,[11] which leads to the assumption that the diagnosis of the disease can be postponed and is dependent on advanced oral manifestations.[8] In most of the cases, pursuit of treatment is neglected by the patient until painful symptomatology or extensive loss of tooth structure are observed.[6]

The restorative treatment of erosive lesions should always be preceded by and concomitant with causal factor intervention.[6,12] The primary goal of the restorative treatment is to reduce symptoms of pain and dentin hypersensitivity, and to restore the dental esthetics and function.[6] In a minimally invasive dentistry era, the therapy of choice should always be the most conservative as possible. Usually, depending on the extent of the lesion, restorations are made with direct or indirect approaches using resin composite or dental ceramics, respectively.[12-14] A systematic review showed inconclusive results regarding the best treatment for the rehabilitation of severe tooth wear.[15] However, there is a consensus for the use of minimally invasive treatment options whenever possible. High-viscosity glass ionomers, especially modern reinforced glass ionomer cements (GIC), present satisfactory mechanical properties.[16] Clinically reinforced GIC showed a similar survival rate compared to resin composite in Class II caries lesions in posterior teeth evaluated up to 10 years.[17] Recent studies have shown that this "new category of high-strength GICs" is suitable to successfully restore load-bearing areas with success after 2 years,[18] after 6 years,[19] and even after 5 years in persons with disabilities.[20] However, the use of GIC in extensive restorations is still a new approach, and the scientific evidence is being built. In addition, a constant use of fluoride materials might minimize the erosive effect of gastric contents on tooth enamel[21]— a valid preventive alternative to the usual replacement of lost dental structure. A recent laboratory study showed that GIC-based materials promoted the lowest tissue loss of enamel adjacent to restoration, when subjected to erosive challenges.[22] Therefore, this material might be the only one able to reduce enamel loss in cases where the causal factors are not well controlled. Moreover, the brushing with fluoridated toothpaste is essential in this process, as a vehicle of fluoride ions to the eroded surface.[6,23]

Although there are clinical cases reporting the use of conventional GIC as an alternative for restoring molar incisor hypomineralisation[24] and dental caries,[25] there is no clinical procedure reported in the literature of multiple tooth restorations with loss of tooth structure due to erosive tooth wear using recent reinforced conventional GICs. The objective of this clinical case report was to present the use of this material for the specific condition of dental erosion, as a possible alternative to the standard treatments used nowadays, with 24-month follow-up.

CLINICAL CASE REPORT

A 21-year-old male patient, complaining of intense sensitivity in the posterior maxillary teeth, sought care

in the university clinics. Clinical examination showed severe wear on maxillary premolar and molar teeth, especially on the palatal surfaces (Figure 1). During anamnesis, the patient reported gastroesophageal reflux disease but did not report any signs of bruxism. Taking these aspects into account, the diagnosis was erosive tooth wear mainly due to intrinsic sources. This condition originated from stomach acid and led to considerable loss of structure and complete loss of the palatal cusps.

The first step was to refer the patient for confirming and treating the medical condition [Gastroesophageal Reflux Disease (GERD)],[26] which was confirmed and treated using medication (proton pump inhibitors) and by lifestyle modifications, such as increasing the number of pillows, avoiding reflux-provoking foods before sleeping (dietary restriction), reducing stress, and the use of chewing gum.[27] After the patient achieved stabilization, he returned for dental treatment. The patient was under medical treatment and counselling during the dental treatment and recall phases.

In addition to the management of the cause of the erosive tooth wear, in order to address the reported pain symptoms and to restore the dental anatomy, the restorative treatment was planned. The final decision regarding the type of treatment followed the shared decision-making model. The dentist provided information regarding treatment options, and then took into consideration patient values and treatment preferences. Finally, the mutual agreement was to perform restorations with the high-viscosity GIC Equia Forte (GC Corporation, Tokyo, Japan), since this material has improved adhesion to a highly smooth, shiny, and polished dentin. Moreover, there was a need for a material with high mechanical strength, since all palatal cusps would be rebuilt and should support occlusal and lateral masticatory loads.

During the first appointment, the initial photographs of the case (Figure 1) were taken, and dental cleaning with ultrasound followed by prophy brush and paste were performed. Impressions of the maxillary and mandibular arches were taken with irreversible hydrocolloid (Avagel, Dentsply, Rio de Janeiro, Brazil). After being disinfected, the mold was poured with plaster stone, following the instructions recommended by the manufacturer. "Wax 7" was used to obtain an occlusal record. The casts were mounted in a semi-adjustable dental articulator (SAA). The maxillary cast was mounted with the aid of a facebow registration. The mandibular cast was then positioned and mounted.

The casts with diagnostic waxing were impressed with condensation silicone (Zetalabor, Zhermack, Badia Polesine, Italy) to produce silicone guides. During the restorative procedure, the GIC setting would take place with the guides in place (Figure 2). The margins of the molds, close to the gingival area, were trimmed for material excess removal during the material setting.

The high-viscosity GIC used in the present study is marketed in capsules for mechanical mixing. That characteristic is important to create a homogeneous mix. This material also was chosen, because it was top-ranked by experts for 18 conventional glass ionomer restorative brands considering mechanical, physical, and chemical properties.[28]

Additional retentive grooves were fabricated along the dentinoenamel junction (DEJ) using #¼ round carbide burs in the mesial and distal surfaces and in the lingual surface (Figure 3), attempting to improve the retention of the GIC. Cotton roll isolation was performed, and each cavity was cleaned with polyacrylic acid (Figure 4) applied for 20 seconds with a micro brush, aiming to clean the surface to receive the restorative material. Washing and drying were performed prior to the material insertion.

The GIC was mechanically mixed for 10 seconds in the CAPSULE MIXER CM-II (GC Corporation), inserted into the silicone guide (Figure 5) and placed in

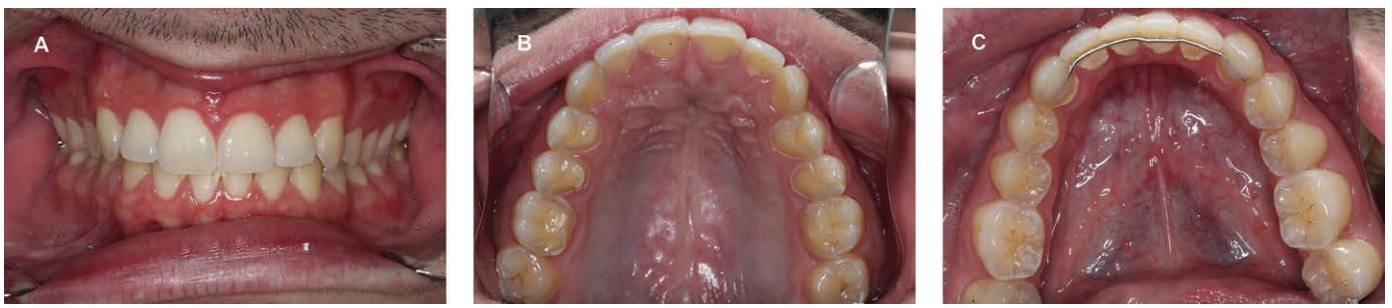

Figure 1. *Initial photographic documentation. (A) Teeth in occlusion (buccal view). (B) Right maxillary premolars and first molar/left maxillary premolars and first molar showing erosive tooth wear (BEWE score 3 for occlusal and palatal surfaces) (occlusal view). (C) Right mandibular premolars and first molar/left mandibular premolars and first molar showing initial erosive wear (BEWE score 1 for occlusal and palatal surfaces) (occlusal view).*

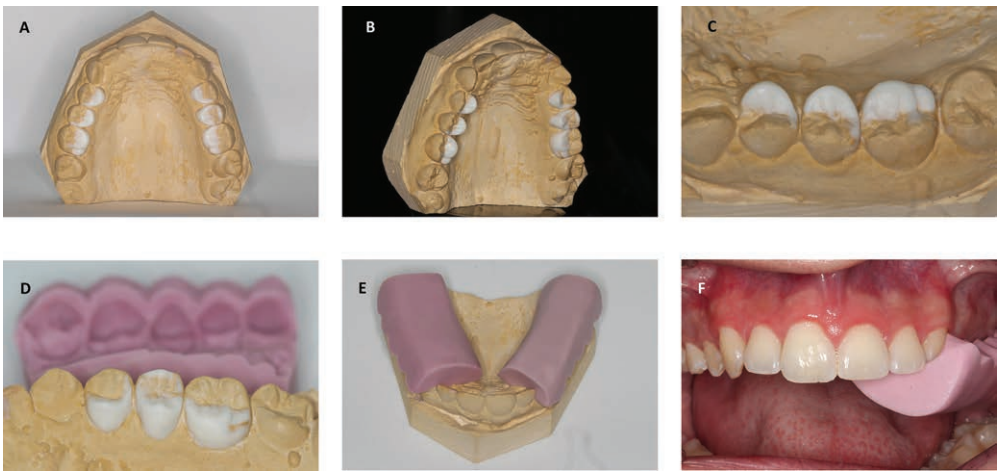

Figure 2. *(A, B, and C) Plaster model with diagnostic waxing. (D and E) Guides constructed with condensation silicone. (F) Silicone guide positioned in the mouth.*

position in the oral cavity (Figure 6). The silicone guide with GIC was held in position under slight pressure for 50 seconds. After the initial hardening of the material (2.5 minutes), the silicone guide was removed, and the excess material was removed with movements from the restoration toward the dental structure (Figure 7) to avoid any possible displacement of the restoration.

After the excess removal with hand instruments, the surface was protected with a resin coating (Equia Coat, GC Corporation), which was further light cured for 20 seconds (Figure 8).

The described technique can be performed on a single tooth or several teeth at once. By restoring several teeth, the waste of restorative material might be reduced. In the present case report, the authors chose to restore three teeth from the same semiarch at the same time, taking into consideration the amount of material present in each Equia Forte capsule. Any excess material, especially within the interproximal region, was removed with the aid of a #12 scalpel blade. Considering the previous interproximal contacts were all in teeth, the procedure was facilitated with minimal excessive material removal.

At the end, occlusal adjustment of the restorations was performed, and the surfaces were again protected with a resin coat. The patient was instructed not to eat in the first 2.5 hours, in order maximize the stabilization of the chemical bond between the GIC and the dental structure (Figure 9).[29]

Immediately after restoration, the patient reported no more sensitivity. The follow-up of restorations was performed monthly. At the 24-month recall, presented in Figure 10, it was possible to observe minor wear on the tips of the cusps, which were rounded, showing the success of the treatment.

DISCUSSION

For extensive posterior restorations, as in cases replacing lost cusps, indirect inlay/onlay restorations are indicated.[30] However, according to the concepts

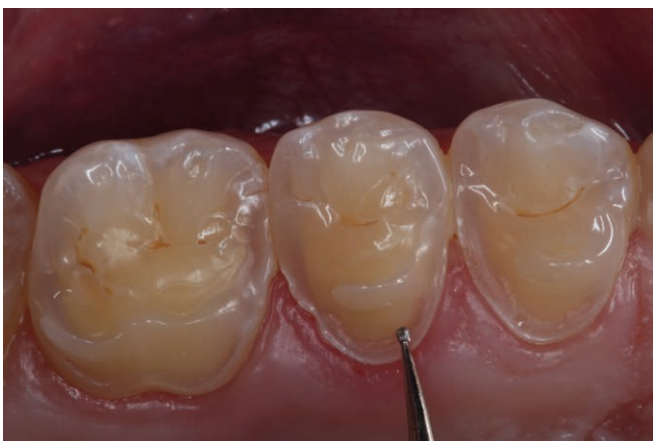

Figure 3. *Additional retention grooves being performed along the lingual surface with small round carbide bur (#1/4).*

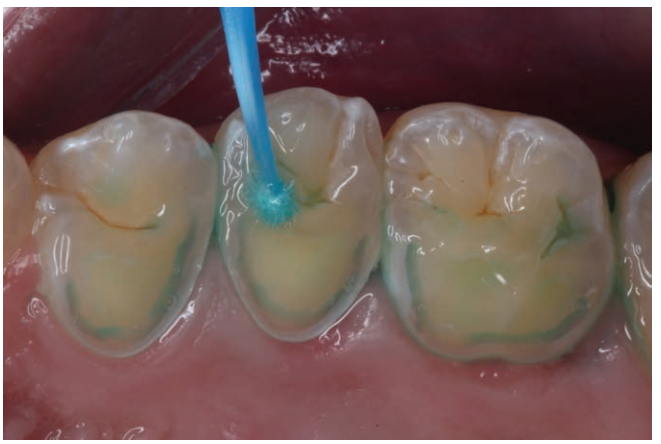

Figure 4. *Polyacrylic acid being applied for 20 seconds with micro brush.*

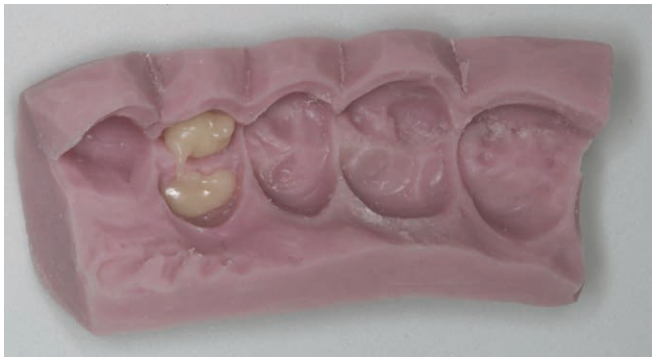

Figure 5. *Glass ionomer cement (GIC) inserted into the silicone guide. All areas of the silicone guide were filled with GIC before its position above teeth.*

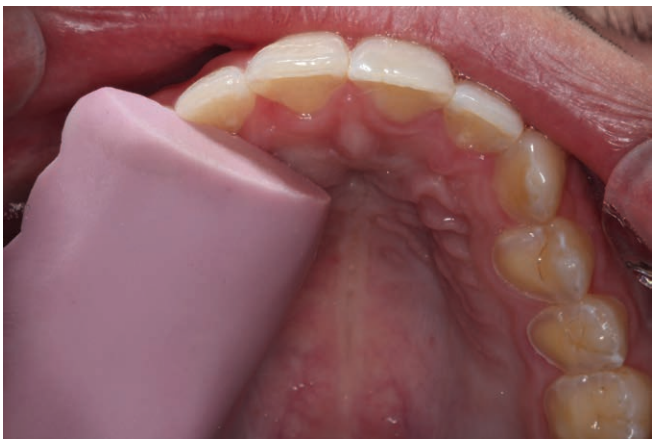

Figure 6. *Silicone guide with glass ionomer cement in place in the mouth.*

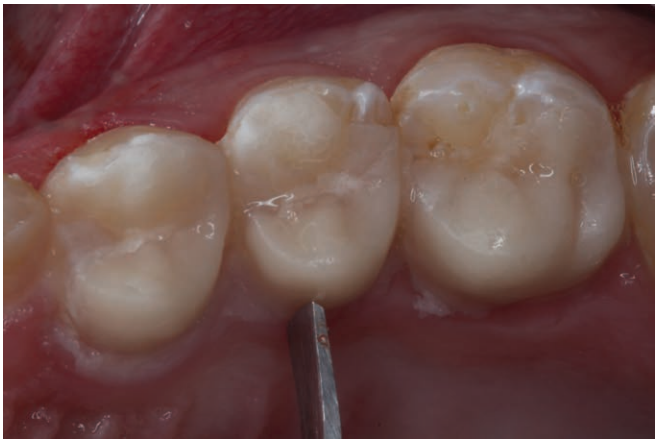

Figure 7. *Removal of restorative material excess.*

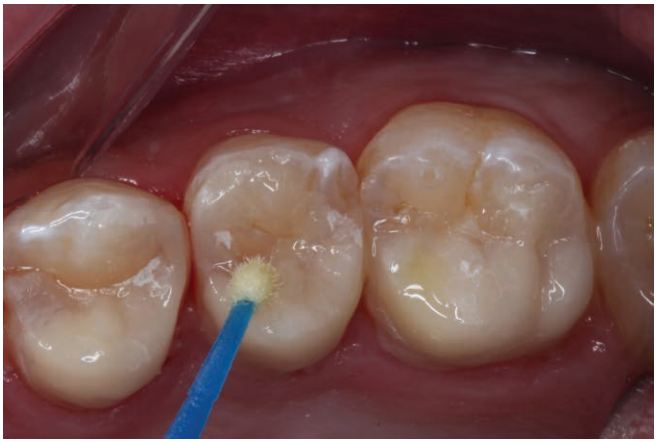

Figure 8. *Surface protection application with micro brush.*

of conservative dentistry, factors such as patient's age and the need for tooth structure preparation should be considered.[31] Following this idea, the main objective is to preserve as much dental structure as possible[31,32] using techniques that allow less or no preparation of the damaged tooth. This is true especially in the case of young patients, since this population presents higher life expectancy and a need for greater longevity of the teeth.[33] Furthermore, by preserving a greater amount of dental tissue, it is possible to extend the well-known restorative cycle that results in tooth death. This cycle, described by Elderton (1988) and Simonsen (1991), concludes that replacing a restoration results in an even larger restoration that will ultimately fail, and no restoration is permanent.[33]

In spite of providing higher resistance to wear, fracture, and discoloration and good marginal adaptation, indirect restorations require preparation and in this case should not be the first restorative option following concepts of minimally invasive dentistry.[33,34]

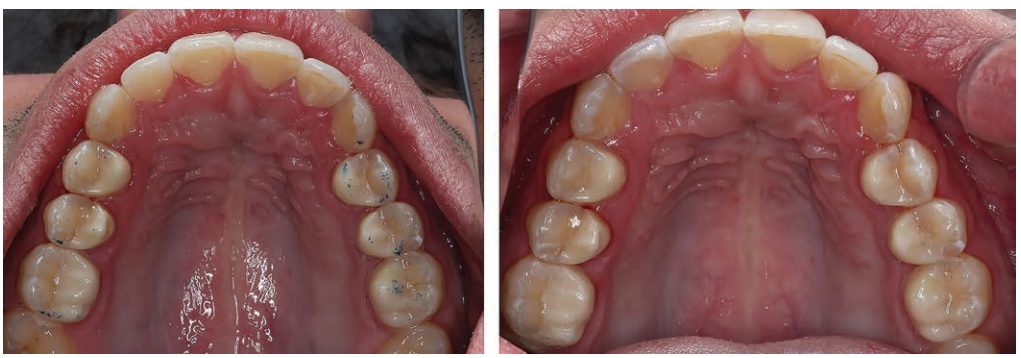

Figure 9. *Occlusal adjustment considering the patient's correct occlusion and vertical dimension.*

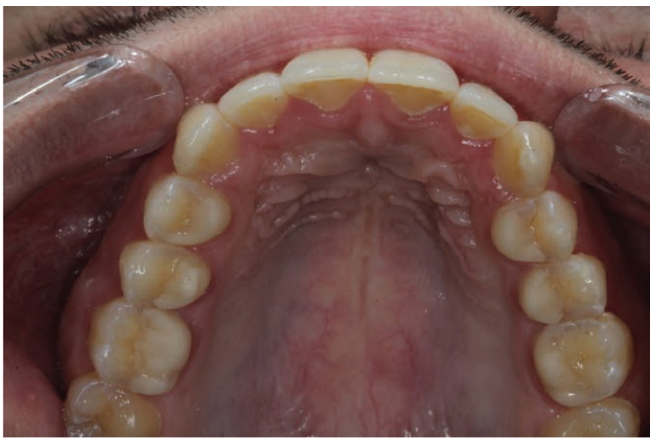

Figure 10. *24-month follow-up of restorations.*

Direct resin composite restorations might not require tooth preparation, which allows a maximum preservation of tooth structure and leads to less cost and time for their construction.[35,36] However, those restorations present some restrictions, such as high technical sensitivity, poor adhesion to dentin (especially when sclerotic), and polymerization shrinkage which can lead to gaps and consequently microleakage.[35-37]

Other options for direct restorations are high-viscosity or resin-modified GIC. These materials have advantages over some resin composite limitations, such as good marginal sealing, absence of polymerization shrinkage stress, a desirable response in cases of erosive lesions, and a satisfactory chemical bond to the dentin.[38] In addition, they are considered bioactive materials,[39] being reservoirs of hydroxyapatite constituents and fluoride. Fluoride release can reduce the effects of erosion, preventing erosive tooth wear occurrence on the enamel adjacent to the restoration in cases where the causal factors are not controlled.[40,41] Clinically, one might consider the quicker restorative approach in comparison to indirect restorations and also the immediate pain control for scenarios such as the one presented.

As adhesion of GIC involves primarily the chelation of enamel and dentin minerals by the carboxyl groups of the polyacids,[42] the creation of mechanical retention in the occlusal-proximal cavities provides additional retention in the first few hours after placement, a critical period because of adhesion being extremely fragile.[43] In this period, external factors not controlled by the technique or the operator, such as the mastication of hard foods, may compromise the restoration. Kemoli and others[44] observed that the success of proximal restorations was significantly influenced by the consistency of the next meal consumed by each child. Children eating harder foods had lower success rates. In addition, the amount of occlusal force applied to the restoration, particularly in the early stages of its maturation, may negatively influence the restorations longevity.[45] The present initial waiting time of 2.5 minutes prior to silicone guide removal used in this case was also performed in another study.[25] That step allowed for initial setting and adequate initial strength was reached. Then, the patient was instructed not to eat, and thus stress the restoration with occlusal forces, for at least 2 hours. These steps might have helped the good results observed in the recall appointments.

The use of silicone guides was considered because of the choice of GICs, providing significant time saving in daily practice. Still, the handling characteristics and setting time of encapsulated GIC would not facilitate the restoration of multiple posterior restorations by free hand, considering the need to create dental anatomy. Thus, the use of the silicone guides also led to restorations with proper anatomy and occlusal contacts. The use of silicone guides with high-viscosity GIC has been previously reported in a case report of Sjögren syndrome for the reconstruction of several teeth with extensive caries lesion.[25]

Improvement in GICs in order to provide greater longevity to multiple surface restorations in permanent teeth has been required. One such improvement is the development of encapsulated high-viscosity materials, which have shown better mechanical properties compared to hand-mixed high-viscosity GICs *in vitro*.[46] Another measure that may increase the longevity is the use of small retention characteristics near the DEJ using a rotating instrument in cases of conventional restorations[47] or manual instruments in cases of atraumatic restorative treatment.[48]

Another important point of the high-viscosity GIC clinical protocol is the application of loaded resin coating (Equia Forte Coat). Because heat is a chemical reaction catalyst, the use of external sources of activation, such as LEDs or halogen lights, significantly reduces the time that the carboxyl content stabilizes, accelerating the setting of the chosen GIC. In this way, there is matured adhesion in approximately 2.5 hours,[43] which certainly is a positive impact on the restoration clinical success.

By presenting this case report, the authors want to emphasize two important aspects regarding the restorative procedure. The lack of resistance of GIC reported in the past is outdated knowledge, considering the recent results in cavities with two or more surfaces in posterior teeth.[17,49] Our 24-month results give reason for optimism when using high-viscosity glass ionomers in previously contraindicated posterior stress-bearing

areas. However, we will continue to monitor the case. Secondly, the use of the silicone guide allowed an adequate outcome regarding the dental anatomy and occlusion, as well as being a time-saving procedure. Moreover, the use of silicone guides facilitated the waiting time of 2.5 minutes with no disturbance to the restoration, like saliva contamination or occlusion by the patient.

CONCLUSIONS

With the restoration success at the 24-month recall, it can be concluded that restoring erosive tooth wear lesions with high-viscosity GIC may be a treatment alternative to restore the quality of life of patients, to restore function and aesthetics, and to remove painful symptoms.

Further clinical research is needed to validate this possible treatment alternative.

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Conflict of Interest

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

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Clinical Research

Combined Bleaching Technique Versus At-home Bleaching—A Single-blind Randomized Controlled Trial

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Clinical Relevance

Combined bleaching with reduction in the application time of 35% hydrogen peroxide and previous use of desensitizer is effective and stable but without reduction in the risk and intensity of dental sensitivity.

SUMMARY

Objective: To compare the efficacy, color stability, and tooth sensitivity (TS) of combined bleaching, using a modified protocol with at-home bleaching.

Methods: Eighty participants were randomized into two groups. In the combined group, a desensitizing gel was applied (10 minutes) prior to in-office bleaching (35% hydrogen peroxide (H_2O_2), 2×15 minute applications) and at-home bleaching (4% H_2O_2, 2×30 minutes for 21 days)

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started the next day. In the at-home group, only the at-home bleaching was performed. Color was recorded at the beginning and postbleaching with two scales (VITA Classical and Bleachedguide) and Easyshade spectrophotometer. The TS was recorded daily with a 0-10 visual analogue scale (VAS) and five-point numeric rating scale (NRS).

Results: A 40% lower risk (RR=1.4; 95% CI 1.1-1.9) was observed in the at-home group. Higher color change and intensity of TS [mean difference 2.3 (95% CI 1.3-3.3) in the VAS] was observed in the first week for the combined group. After the end of the protocol, a bleaching degree was detected for both groups, with no significant difference between both groups (p>0.05).

Conclusion: The combined group produced a slightly higher degree of color change than at-home bleaching but with a higher risk and intensity of TS.

INTRODUCTION

Dental bleaching has become increasingly sought by patients to improve the aesthetics of darkened teeth and the harmony of the smile.[1,2] For such purposes, there are two dentist-supervised categories—at-home and in-office, and both can be combined in so-called combined bleaching.[3]

At-home dental bleaching involves the use of individual trays with carbamide peroxide (CP) or low concentration hydrogen peroxide (H_2O_2) gels[4-6] It has the advantages of a lower risk and intensity of tooth sensitivity (TS),[7-9] and shorter chair time, although satisfactory results are obtained with 2-4 weeks of treatment.[10] In-office dental bleaching involves higher concentrations of H_2O_2[3,11-13] and, therefore, can provide faster results than at-home bleaching, with the disadvantage of generating a higher risk and intensity of TS during or after the bleaching session.[8,14,15]

H_2O_2 has a low molecular mass, which favors its penetration through the enamel, reaching the dentin and pulp tissue.[16] Upon penetrating these structures, their oxidizing components rapidly diffuse through the tissues, reaching the chemosensitive ion channel (TRPA1), which possibly directly activates the intradental nerve via TRPA1.[17] The first damaged cell is the odontoblast that is attached to the roof of the pulp chamber. When these cells undergo the action of oxidizing components of H_2O_2, they collapse due to oxidative stress but are subsequently replaced by newly differentiated mesenchymal cells.[18] However, all of these reactions lead to an inflammatory process that is directly responsible for generation of bleaching-induced TS.[17]

Although at-home bleaching with 10% CP is considered the gold standard,[19-21] the need for a long-lasting treatment when using in-office bleaching has led dentists and patients to seek a safer and faster dental bleaching protocol. A common clinical practice is the association of both in-office and at-home protocols to potentialize the bleaching effect[4] and maintain long-term color stability.[6,22]

In this context, in-office bleaching is performed during the first session to provide an initial "jump-start" bleaching effect.[3,6] Subsequently, the patient receives an individual bleaching tray to perform home bleaching, until the desired shade is obtained.[4,22] With the accomplishment of this technique, some authors have reported a reduction of the risk of TS[23] with satisfactory results.[4,22,24]

In an attempt to reduce TS[3,25] some alternatives have been proposed, such as a reduction of bleaching gel concentration,[26] the use of bleaching agents for shorter periods of time,[27,28] the use of drugs,[29-33] and topical use of desensitizing agents before or after bleaching.[34-38] However, studies combining drug use with dental bleaching reported no reduction in TS.[29-33] Among the topical desensitizing agents used are 2% sodium fluoride, 2-hydroxymethyl methacrylate with glutaraldehyde, and 5% potassium nitrate.[35,36,39] These topical agents have been reported as effective methods to reduce the risk and intensity of TS when applied before[35,37] or after bleaching.[38,40] Also, for in-office bleaching, reducing the number of applications of 35% H_2O_2 minimized the intensity of TS, as reported in the clinical trial published by Kose and others,[28] who found that two applications of 15 minutes was as effective as three applications of 15 minutes, but with a reduced intensity of TS.

Although these alternatives have already been tested for in-office bleaching, it is still unknown if they can bring similar benefits when combined bleaching is used. The association of more than one attempt to reduce bleaching-induced TS may allow better acceptability in terms of reduced side effects. Therefore, the objective of this study was to compare the efficacy, color stability, and TS of a combined bleaching technique with a modified protocol to reduce bleaching-induced TS in adults with at-home bleaching.

METHODS AND MATERIALS

Trial Design, Settings, and Locations of Data Collection

This study was a randomized, parallel, single blind, and equivalence trial. Only the evaluator was masked to patient group assignment. The study was performed

from October 10, 2016, to June 7, 2017, in the clinics of the School of Dentistry State of the University of Ponta Grossa.

Recruitment

Participants were recruited through written advertisements placed on the university building walls. All participants signed an informed consent form before being enrolled in the study.

Eligibility Criteria

Volunteers included in the clinical trial were at least 18 years old, had good general and oral health, and did not report any type of TS. The volunteers were required to have six caries-free and restoration-free maxillary anterior teeth and healthy periodontal tissues. The central incisors had to be shade A2 or darker, as judged by comparison with a value-oriented shade guide (VITA Classical, Vita Zahnfabrik, Bad Säckingen, Germany).

Volunteers with anterior restorations or dental prosthesis, orthodontic apparatus, or severe internal tooth discoloration (tetracycline stains, fluorosis, pulpless teeth) were not included in the study. In addition, pregnant and lactating women, volunteers with any other pathology that could cause sensitivity (such as recession, dentinal exposure, visible cracks in teeth), taking anti-inflammatory or analgesic drugs, who smoked or had bruxism, or volunteers who had undergone tooth-bleaching procedures were excluded.

Sample Size Calculation

The absolute risk of bleaching-induced TS was previously reported to be 85%[6] when using 35% H_2O_2 associated with 10% carbamide peroxide. Considering an equivalence limit of 25% in the rate of bleaching-induced TS, a minimum of 80 volunteers would be required to detect such difference, if it exists, with a power of 90% and an alpha of 5%.

Randomization and Allocation Concealment

A third person who was not involved in implementation and evaluation steps performed a blocked randomization process (blocks of 2 and 4) using the website www.sealedenvelope.com. Block randomization was performed to allow groups with equivalent sample size. Details of the random sequence were recorded on cards, which were placed in sequentially numbered, opaque, and sealed envelopes.

The information contained in the envelope determined the group to which the volunteer would be assigned. Once the participant was eligible for the procedure and completed all baseline assessments, the allocation assignment was revealed by the third person opening this envelope immediately after implementation.

Study Intervention

Alginate impressions of each subject's maxillary and mandibular arch were made and filled with dental stone. No block-out material was applied to the labial surfaces of the stone model teeth. A 1 mm soft, acetate vinyl material provided by the manufacturer (FGM, Joinville, SC, Brazil) was used to fabricate the custom-fitted tray to hold the at-home bleaching gel. The bleaching tray was trimmed 1 mm beyond the gingival margin.

In the combined bleaching group, volunteers were submitted to a single clinical session of in-office bleaching with 35% H_2O_2 gel (Whiteness HP Maxx, FGM). A lip retractor (ArcFlex, FGM) was placed and a desensitizing gel based on potassium nitrate and sodium fluoride (Desensibilize KF 2%, FGM) was applied and left undisturbed for 10 minutes. The gel was removed with a disposable aspirator, and the teeth were cleaned with gauze. Then, the gingival tissue of the teeth to be bleached was isolated using a light-cured resin dam (Top Dam, FGM), and each tooth was light cured for 20 seconds (Radii Cal, SDI, Victoria, Australia). The in-office bleaching gel was applied in two 15 minute applications[28] and not in three 15 minutes applications, as recommended by the manufacturer.

Then, the participants received the bleaching tray and the 4% H_2O_2 gel (White Class with Calcium 4%, FGM). Participants were instructed to start the at-home bleaching the day after the in-office session using the bleaching tray with gel daily for 30 minutes, for 21 days.

In the at-home bleaching group (control), volunteers only performed the at-home bleaching with the 4% H_2O_2 gel (White Class with Calcium 4%, FGM) following the same protocol as described above.

Outcomes

Tooth Sensitivity—Tooth sensitivity (TS) in the combined bleaching group was evaluated immediately after the bleaching and during the 21 days of at-home bleaching. In the at-home group, TS was evaluated daily during the 21 days of treatment. In both the groups, TS was assessed using a 0–10 visual analogue scale (VAS) and five-point numeric rating scale (NRS).

The VAS is a 10-cm horizontal line with scores of 0 and 10 at the ends, with 0 meaning no sensitivity and 10 meaning severe TS. The patient marked the TS intensity with a vertical line across the horizontal line

of the scale. Then, the distance in millimeters from the zero end was measured with the aid of a millimeter ruler.[31,32] Using the five-point NRS, where 0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe, the participants were instructed to indicate the numerical value of the degree of sensitivity.[6,37,41] During the 21-day treatment period, patients scored the intensity of TS once daily. If the patient did not have pain, he or she was instructed to mark a zero on both the scales.

If the participant scored 0 (no sensitivity) in all time assessments, he or she was considered to be insensitive to the bleaching protocol. In all other circumstances, the participants were considered to have bleaching-induced TS. This dichotomization allowed us to calculate the absolute risk of TS, which is the percentage of patients who reported TS at least once during treatment.

To calculate the TS intensity, we took the worst score from the NRS scale and the highest numerical value obtained in the VAS scale reported by each patient so that only a single value per patient was taken from the whole bleaching period.

Color Change—Two experienced and calibrated dentists (kappa statistic greater than 80% after previous calibration) who were not involved in the randomization procedures performed assessments at baseline, after 1, 2, and 3 weeks of treatment, and 1 week, 1 month, and 6 months after bleaching for both the groups. The color of the patient's teeth was not evaluated immediately after the in-office bleaching session, to avoid the effects of dehydration and demineralization on color measurements.

The subjective color evaluation was performed with a VITA Classical shade guide (VITA Classical, Vita Zahnfabrik) and VITA Bleachedguide 3D-MASTER shade guide (Vita Zahnfabrik). An objective color evaluation was also performed using a VITA Easyshade (VITA Zahnfabrik, Bad Säckingen, Germany) spectrophotometer.

The VITA Classical shade guide (VITA Classical, Vita Zahnfabrik) is composed of 16 color guide tabs organized from the highest (B1) to the lowest value (C4). The VITA Bleachedguide 3D-MASTER (Vita Zahnfabrik) scale contains clearer color tabs already organized from the highest (0M1) to the lowest value (5M3).

The area of interest for the measurement of tooth color matching was the middle-third of the facial surface of the anterior central incisors. Color changes were calculated from the beginning of the active phase up to the individual recall times by calculating the change in the number of shade guide units (ΔSGU), which occurred toward the lighter end of the value-oriented list of shade tabs. In the event of disagreement between the examiners during shade evaluation, a consensus was reached.

Objective color evaluation was performed using a VITA Easyshade spectrophotometer (Vita Zahnfabrik) according to the CIELab system. In order to standardize the region of the tooth that was measured, the upper arch of all patients was impressed with condensation silicone (Perfil Cub, Vigodent, Rio de Janeiro, Brazil) for the preparation of a guide. The guide was perforated in the vestibular region in the middle-third of the right upper central incisor with the aid of a 5-mm diameter circular scalpel (Biopsy Punch, Miltex, York, Pennsylvania, USA) similar to the active tip of the appliance. Color change was evaluated by the researchers at the same time as assessments reported for the shade guides. The spectrophotometer was always calibrated daily before measurements.

Color change in ΔE was determined using the CIELab* parameters[42] L^*, a^*, and b^*, where L^* represents brightness ranging from 0 (black) to 100 (white), and a^* and b^* represent the chromatic axes, where a^* is the measure along the red–green axis, and b^* is measured along the yellow–blue axis.

The color variation (ΔE^*_{ab} and ΔE_{00})[43,44] before and after the treatment was calculated by the formulas: $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)2]^{1/2}$ and ΔE_{00} (CIEDE2000) = $[(\Delta L/\mathrm{KlSL})^2 + (\Delta C/\mathrm{kCSC})^2 + (\Delta H/\mathrm{kHSH})^2 + \mathrm{RT}\,(\Delta C^*\Delta H/\mathrm{SC^*SH})^{1/2}$.

Statistical Analysis

Data from 80 patients were used in this study, according to the intention-to-treat analysis.[45] In case of missing data due to nonattendance at the recall visits, data from the last observation were carried forward. The absolute risk of TS in both the groups was compared using Fisher's exact test at a 5% level of significance. The relative risk as well as the 95% confidence interval was also calculated.

The TS intensity data obtained with the NRS scale were analyzed using Mann–Whitney (NRS). For this scale, comparison between assessment times within each group were performed using the Friedman test. Data of TS intensity obtained with the VAS scale were analysed with a two-way ANOVA with repeated measures.

For each instrument of color assessment (ΔSGU in both scales, ΔE^*_{ab} and ΔE_{00}), the color change of groups were compared using a two-way repeated measures ANOVA (groups *vs.* assessment time). Tukey test was used for pairwise comparisons. In all statistical tests, the significance level was 0.05. We performed all the analyses by using the software SigmaPlot version 11.0 (Systat Software).

RESULTS

Characteristics of Included Participants

A total of 121 volunteers were examined in a dental chair to check if they met the inclusion and exclusion criteria. A total of 80 patients were included in this clinical study (Figure 1). Seventy-eight patients completed the bleaching protocols of this study and attended the 1-week, 1-month, and 6-month recalls. Only two patients discontinued intervention.

Similar baseline features were observed between the two study groups. The baseline color of the participants in SGU was 5.7 ± 1.4 for the combined bleaching group and 6.0 ± 1.7 for the at-home bleaching group. The mean age (years) of the participants was 23.2 ± 4.9 for the combined bleaching group and 22.9 ± 4.8 for the at-home bleaching group. Females represented 62.5% of the combined bleaching group and 52.5% of the at-home bleaching group.

Color Change

For the VITA Classical shade guide (Table 1), the mean difference (95% CI) for the groups was 0.4 (−0.3-1.0), while for the spectrophotometer the mean difference for the ΔE^*_{ab} was −0.4 (−2.6-1.8; Table 2) and ΔE_{00} was −0.5 (−2.13-1.13; Table 3). For all these measures of color evaluation, only the main factor time ($p<0.001$) was statistically significant, meaning that a significant color change occurred over time irrespective of the group. At the end of the bleaching protocol, bleaching of approximately 4 SGU was detected; a ΔE^*_{ab} of 9.0 units and ΔE_{00} of approximately 7.0 units were detected for both the groups (Tables 1-3).

In the VITA Bleachedguide shade guide, the main factors time ($p<0.001$) and group ($p=0.04$) were statistically significant. Bleaching increased over time for both groups, and a statistically greater color change was observed for the combined bleaching group. The mean difference of color change was 1.2 (0.0-2.4), which can be clinically detected by a calibrated operator (Table 4).

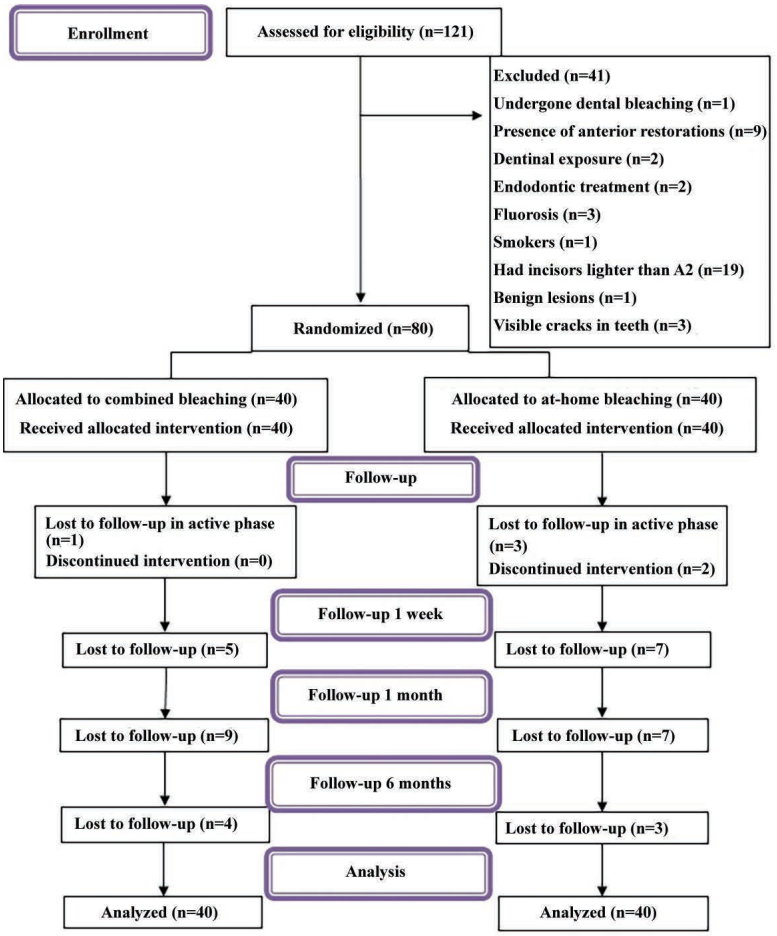

Figure 1. Flow diagram of the clinical trial, including detailed information on the excluded participants.

Table 1: *Means and Standard Deviations of ΔSGU Values in the Vita Classical Scale, For the Two Groups, at the Different Time Assessments*[a]

Periods	Combined Bleaching	At-home Bleaching	Main Factor Time	Mean Difference (95% CI)
Baseline vs 1st week	3.7 ± 1.6	3.0 ± 1.7	3.4 ± 1.6 a	0.7 (–0.0-1.4)
Baseline vs 2nd week	4.1 ± 1.4	3.6 ± 1.4	3.8 ± 1.4 b	0.5 (–0.1-1.1)
Baseline vs 3rd week	4.4 ± 1.3	4.4 ± 1.5	4.4 ± 1.4 c	0.0 (–0.6-0.6)
Baseline vs 1st week after	4.4 ± 1.3	4.0 ± 1.4	4.2 ± 1.4 c	0.4 (–0.2–1.0)
Baseline vs 1 month after	4.4 ± 1.3	4.0 ± 1.3	4.2 ± 1.4 c	0.4 (0.2-1.0)
Baseline vs 6 months after	4.4 ± 1.3	3.9 ± 1.7	4.1 ± 1.5 c	0.5 (–02-1.2)
Main factor group	4.2 ± 1.4 A	3.8 ± 1.5 A	—	0.4 (–0.3-1.0)

Abbreviations: CI, confidence interval; SGU, shade guide units.
[a] *Uppercase letters indicate statistical similarity between groups and lowercase letters indicate statistical similarity among times of assessment.*

Table 2: *Means and Standard Deviations of the ΔE Values Obtained by the Vita Easyshade Spectrophotometer, for the Two Groups, at the Different Time Assessments*[a]

Periods	Combined Bleaching	At-home Bleaching	Main Factor Time	Mean Difference (95% CI)
Baseline vs 1st week	8.8 ± 6.2	8.8 ± 4.0	8.8 ± 5.1 a	0 (–2.3-2.3)
Baseline vs 2nd week	8.8 ± 4.8	8.6 ± 4.8	8.7 ± 4.8 ab	0.2 (–1.9-2.3)
Baseline vs 3rd week	10.1 ± 5.1	9.8 ± 5.2	9.9 ± 5.1 ab	0.3 (–2-2.6)
Baseline vs 1st week after	8.4 ± 4.0	8.8 ± 4.7	8.6 ± 4.3 ab	–0.4 (–2.3-1.5)
Baseline vs 1 month after	9.7 ± 5.2	12.0 ± 6.4	10.8 ± 5.8 b	–2.3 (–5-0.3)
Baseline vs 6 months after	9.3 ± 4.6	9.6 ± 4.6	9.4 ± 4.6 b	–0.3 (–2.3-1.8)
Main factor group	9.2 ± 5.0 A	9.6 ± 4.9 A	—	–0.4 (–2.6-1.8)

Abbreviations: CI, confidence interval.
[a] *Uppercase letters indicate statistical similarity between groups and lowercase letters indicate statistical similarity among time assessments.*

Table 3: *Means and Standard Deviations of the ΔE_{00} Values Obtained by the Vita Easyshade Spectrophotometer, for the Two Groups, at the Different Time Assessments*[a]

Periods	Combined Bleaching	At-home Bleaching	Mean Difference (95% CI)	Mean Difference (95% CI)
Baseline vs 1st week	6.5 ± 4.4	6.5 ± 3.0	6.5 ± 3.7 a	0 (–1.68-1.68)
Baseline vs 2nd week	6.3 ± 3.5	6.5 ± 3.6	6.4 ± 3.5 ab	–0.2 (–1.78-1.38)
Baseline vs 3rd week	7.1 ± 3.6	7.1 ± 3.8	7.1 ± 3.7 b	0 (–1.65-1.65)
Baseline vs 1st week after	6.1 ± 3.1	6.5 ± 3.3	6.3 ± 3.2 ab	–0.4 (–1.83-1.03)
Baseline vs 1 month after	7.0 ± 3.8	8.9 ± 5.0	7.9 ± 4.4 b	–1.9 (–3.88-0.08)
Baseline vs 6 months after	6.7 ± 3.2	6.9 ± 3.5	6.8 ± 3.4 ab	–0.2 (–1.69-1.29)
Main factor group	6.6 ± 3.6 A	7.1 ± 3.7 A	—	–0.5 (–2.13-1.13)

Abbreviation: CI, confidence interval.
[a] *Uppercase letters indicate statistical similarity between groups and lowercase letters indicate statistical similarity among time assessments.*

Table 4: *Mean and Standard Deviations of ΔSGU Values on the Vita Bleachedguide Shade Guide, for the Two Groups, in the Different Evaluation Periods*[a]

Periods	Combined Bleaching	At-home Bleaching	Main Factor Time	Mean Difference (95% CI)
Baseline vs 1st week	4.5 ± 2.9	2.7 ± 2.5	3.6 ± 2.7 a	1.8 (0.6-3.0)
Baseline vs 2nd week	5.5 ± 2.6	3.7 ± 2.4	4.6 ± 2.5 b	1.8 (0.7-2.3)
Baseline vs 3rd week	6.0 ± 3.2	4.8 ± 2.8	5.4 ± 3.0 c	1.2 (–0.1-2.5)
Baseline vs 1 week after	6.0 ± 3.3	4.8 ± 2.8	5.4 ± 3.0 c	1.2 (–0.2-2.6)
Baseline vs 1 month after	5.7 ± 2.8	5.0 ± 2.0	5.4 ± 2.4 c	0.7 (–0.4-1.8)
Baseline vs 6 month after	5.5 ± 2.8	4.8 ± 2.8	5.1 ± 2.8 c	0.7 (–0.5-1.9)
Main factor time	5.5 ± 2.9 A	4.3 ± 2.5 B	—	1.2 (0-2.4)

Abbreviations: CI, confidence interval;SGU, shade guide units.
[a] Uppercase letters indicate statistical similarity between groups and lowercase letters indicate statistical similarity among time assessments.

Tooth Sensitivity (TS)

A significantly higher risk of TS was observed for the combined bleaching (90%; 95% CI 77-96) than at-home bleaching (63%; 95% CI 47-76) (Table 5, p=0.008). Regarding TS intensity, a statistical difference between the groups in the first week of bleaching was detected (Tables 6 and 7, p<0.001).

TS intensity was higher for the combined bleaching group in the first week of bleaching. The magnitude of the difference in pain intensity was 2.3 (95% CI 1.3-3.3) VAS units (Table 7). After the first week, no significant difference in TS intensity between the groups was detected. In general, TS intensity decreased significantly over time for both groups (Tables 6 and 7, p<0.05).

Table 5: *Comparison of the Number of Patients Who Reported TS During Bleaching Treatment with Absolute and Relative Risks*[a]

Treatment	Tooth Sensitivity (Number of Patients)		Absolute Risk (95% CI)	Relative Risk (95% CI)
	Yes	No		
Combined bleaching	36	4	90 (77-96)	1.4 (1.1-1.9)
At-home bleaching	25	15	63 (47-76)	

Abbreviations: CI, confidence interval; TS, tooth sensitivity.
[a] Statistical comparison between groups was performed with Fisher exact test (p=0.008).

DISCUSSION

In this study, a significant statistical difference was found for the risk and intensity of TS reported by the volunteers among the groups evaluated. At-home bleaching produced a lower risk and intensity of TS, when compared to the associated bleaching, being in agreement with other studies in the literature.[6,9,46] This difference may be directly correlated with the low concentration of H_2O_2 used in the at-home technique. By using low-concentration products, a smaller amount of H_2O_2 reaches the pulp chamber within the time of application.[19,47]

The study by Soares and others[48] demonstrated reduced aggression of the pulp cells when bleaching was performed with low-concentration products. The at-home bleaching agent also contains potassium nitrate and sodium fluoride as desensitizing agents, as

Table 6: *Medians (Interquartile Range) of TS Intensity Obtained with the NRS Scale*[a]

Periods	NRS (0-4)		*p*-Value (*)
	Combined Bleaching	At-home	
1st week	2 (1/3) A	1 (0/1) A	<0.001
2nd week	0 (0/0) B	0 (0/1) A	0.28
3rd week	0 (0/0) B	0 (0/2) A	0.21

Abbreviations: NRS, numeric rating scale.
[a] Statistical comparison between groups were performed with Mann–Whitney test (*). Comparison of the different assessment times within each group were performed with the Friedman test (p<0.05), and significant differences are represented by different uppercase letters.

Table 7: *Means, Standard Deviations and Mean Difference (95% Confidence Interval [CI]) of TS Intensity Obtained with the VAS Scale*[a]

Periods	VAS (0-10)		Mean Difference (95% CI)	*p*-Value
	Combined Bleaching	At-home		
1st week	3.2 ± 3.0 A	0.9 ± 1.3 A	2.3 (1.3-3.3)	<0.001
2nd week	0.4 ± 0.7 B	0.8 ± 1.7 AB	–0.4 (–1.0-0.2)	0.48
3rd week	0.3 ± 0.5 B	0.5 ± 1.0 B	–0.2 (–0.6-0.2)	0.38

Abbreviations: VAS, visual analogue scale.

[a]*Mann–Whitney test. The assessment times within each group were compared with the Friedman test (p<0.05), and significant differences are represented by different uppercase letters.*

well as calcium,[49] which has been claimed to reduce the risk and intensity of TS of at-home products.

The absolute risk of TS from at-home bleaching in this study was 62%. Other studies in the literature evaluated similar concentrations of H_2O_2, such as those of Myers and others,[50] which found an absolute risk of TS of 48%, and Chemin and others,[26] which reported TS risks varying from 25% to 54% with the same at-home bleaching protocol as used in this study. The low absolute risk and intensity of TS observed in the at-home bleaching of this clinical trial appear to be further evidence of the correlation between the H_2O_2 concentration and the risk and intensity of TS. Studies comparing the risk and intensity of TS in at-home bleaching versus in-office bleaching also reported favorable results for the at-home protocol.[3,9,35,51,52]

The higher risk and intensity of TS of in-office bleaching[6] explains why the combined bleaching of this study showed a higher risk and intensity of TS than the at-home protocol. We expected that by using only two 15 minute applications, instead of the recommended three 15 minute applications, and the preliminary application of potassium nitrate, bleaching-induced TS caused by in-office bleaching could be minimized, but these alternatives were shown to be unfruitful.

In the combined bleaching, we used a high-concentration 35% H_2O_2, which has been attributed to a higher risk and intensity of TS than in-office bleaching with 20% H_2O_2.[6] Perhaps the high concentration of H_2O_2 in the combined bleaching protocol followed by the daily use of the tray in the at-home protocol overrode the benefits achieved by the reduction in the number of applications and prior use of a desensitizing agent. Additionally, recent well-designed and well-powered randomized clinical trials have reported that application of potassium nitrate before and after bleaching does not have the benefits otherwise shown in earlier and small clinical trials.[35,53,54] Perhaps false positive results were obtained in these earlier trials, which were not confirmed by other studies.[34,37,39]

For color change evaluation, we used three different instruments. The VITA Classical shade guide is widely used in clinical trials of bleaching and, therefore, allows comparison of results with previous clinical studies. Although it consists of a valid method, with good reliability to differentiate between dark and light colors,[19] it was not designed for bleaching studies and lacks uniformity between different color tabs, leading to some overlaps between similar colors. This reduces the sensitivity of this tool to detect color changes.[55,56] Another instrument used was the VITA Bleachedguide 3D-MASTER scale, developed for the purposes of dental bleaching evaluation. Different from the VITA Classical, this new shade guide contains shades lighter than B1, which expands its sensitivity to detect subtle differences in the bleaching process.[32,57,58] The VITA Easyshade spectrophotometer apparatus provides an objective and consistent assessment of color change that is less affected by observer training and variability.[55,59]

In the present study, in addition to the conventional CIELab 76 system (ΔE^*_{ab}), other systems were used including the CIEDE2000 system (ΔE_{00}).[60,61]. This new system allowed for better adjustment than the CIELab formula did in estimating the visual perception of color and allowed a better evaluation of the color-difference thresholds.[62,63] Although CIEDE2000 is more advanced, no difference among groups or assessment times were observed when CIELab 76 system or CIEDE2000 system were used. Actually, as the vast majority of previously published bleaching studies still report their findings using ΔE^*_{ab}, it is important to ensure that the present data will be able to be compared with the previous literature. This justified the presentation of data using both formula (ΔE^*_{ab}, and ΔE_{00}).

It is worth mentioning that, for all spectrophotometer measurements, a colored impression material was used

as a guide to assure that the spectophotometer tip was put in the same position and in intimate contact with the dental surface during all color measurements. However, some studies used a transparent impression material, mainly because the authors expected a color interference from the colored impression material in the color measurement.[3,41,46,51] The colored impression material used in the present study has been used in several studies, and none of them describe a color interference.[2,6,26,28,32,53,54] However, to the extent of the author's knowledge, no study was found evaluating this hypothesis. Therefore, future studies need to be done regarding this topic.

In the present study, the VITA Bleachedguide 3D-MASTER shade guide was the only tool that detected a subtle difference between groups throughout the dental bleaching period. A difference in color change of approximately 1.2 units of the 3D-MASTER Bleachedguide was detected. All patients were exposed to 4% H_2O_2 30 minutes daily for 21 days; however, patients from the combined group received an extra 30 minute application of 35% hydrogen in the first clinical appointment. This may be the reason why a higher degree of bleaching was detected for the combined protocol. Had the at-home bleaching continued for an extra week, similar results might have been obtained.

Combined bleaching was suggested to potentiate the bleaching effect[4] and improve color stability.[6,22] The efficacy of bleaching was detected using the three instruments used for color evaluation, according to previous studies in the literature, both for at-home bleaching[26,48,64-66] and combined bleaching.[6,9,46] Although a small but significant higher degree of bleaching was detected using the 3D-MASTER Bleachedguide, this also reduced the risk of pain.

Further well-designed and well-powered randomized clinical trials of combined bleaching should be conducted in order to find a protocol that provides faster bleaching and low risk and intensity of TS.

CONCLUSIONS

Both the combined and at-home bleaching protocols yielded an effective and stable color change 6 months posttreatment. The combined bleaching not only showed a slightly higher degree of bleaching but also a higher risk and intensity of TS.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies of Committee for the Protection of Human Participants of the State University of Ponta Grossa. The approval code issued for this study is protocol number 1.879.340. It was registered in the Brazilian Clinical Trials Registry (REBEC) under identification number RBR-9GNHTH.

Conflict of Interest

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

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Impedance Spectroscopy as a Tool for the Detection of Occlusal Noncavitated Carious Lesions

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Clinical Relevance

The diagnosis of caries in its early stages is important to allow minimally invasive treatments. Impedance spectroscopy may be useful for the detection of occlusal caries.

SUMMARY

A total 302 teeth (148 molars and 154 premolars) corresponding to 152 patients aged ≥18 years were evaluated for caries using the ICDAS (International Caries Detection and Assessment System), fluorescence (DD, DIAGNOdent) and electrical impedance (IMS, CarieScan PRO) systems. Fissurotomy and intraoral radiographs were used as the gold standard. Accordingly, 27.5% (n=84) of the teeth were classified as sound, while 26.9% (n=81) had enamel involvement and 45.6% (n=138) presented carious lesions reaching the dentin. Sensitivity (Se), specificity (Sp), and the area under the curve (AUC) were, respectively, 90.7%, 87.8%, and 0.954 (IMS); 92.4%, 92.7%, and 0.954 (DD); and 79.0%, 72.3%, and 0.756 (ICDAS). With regard to Se and Sp, there were significant differences between ICDAS and DD (p<0.001) and between ICDAS and IMS (p=0.01), but not between IMS and DD (p=0.07). In relation to AUC, there were significant differences between ICDAS and DD (p<0.001), and between ICDAS and IMS (p<0.001), but not between IMS and DD (p>0.05). The correlations between fissurotomy and each method were 88.7% (IMS), 89.7% (DD), and 77.1% (ICDAS). Within the limitations of this study, clinically, the electrical system is not useful for differentiating between sound teeth and truly incipient caries lesions by itself. The fluorescence or electrical systems are

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recommended with the ICDAS to detect carious lesions in their early stages.

INTRODUCTION

The early detection of occlusal caries can be complicated when the lesions are not cavitated.[1] Among other factors, this is due to the complex anatomy of the molars and premolars, and the use of fluoride products, which can increase enamel surface resistance and mask dentinal lesions.[2] In the last 20 years, many studies have attempted to evaluate the detection capacity of different systems, based mainly on fluorescence,[3-8] which involves the absorption of light of a short wavelength and the emission of light of a longer wavelength. The detection of caries, when the lesions are noncavitated and only affect the enamel layer, allows for more conservative management,[9-12] such as fluoride therapy[13] or ozone treatment,[11] which, in turn, implies less-expensive management and the preservation of more dental tissue, thereby, improving patient comfort and quality of life.[12]

The original visual criteria established by Ekstrand and others[14] are no longer regarded as suitable, since they do not contemplate all the stages of caries progression.[8,15] As a result, new visual criteria are being introduced. In this regard, the International Caries Detection and Assessment System (ICDAS) was presented in 2002,[15] allowing assessment of the caries process in each of its stages and determining caries lesion activity status.[16] The ICDAS is a clinical scoring system based on visual examination, optionally aided by a ball-ended probe. The system classifies the stages of the caries process based on histological extent and activity. Seven stages from ''sound'' to ''extensive distinct cavity with visible dentin'' are distinguished, based on the visual appearance of the tooth surface. Different studies support the validity of this system, finding it to be reproducible and precise.[17,18] However, there is great variation in the clinician-based diagnosis of hidden caries, and in this regard technological advances could contribute to improving the detection and diagnosis of caries in its early stages.[19,20] The ICDAS can be used in epidemiological studies, public health research, clinical research, clinical practice, and dental education. It is necessary to train and calibrate the examiners to ensure that the results obtained are comparable among different clinicians and useful, for example, in epidemiological studies.[21]

One of the existing fluorescence-based systems involving red laser (655 nm) measures the differences in fluorescence emitted by the healthy tissues and caries.[22,23] Part of the irradiated light is reemitted by bacterial porphyrins[2] as fluorescence within the infrared spectrum and can be quantified. This technique was introduced in 1998, and its detection ability has been extensively evaluated. Most reports are *in vitro* studies,[2,24-26] though some *in vivo* studies have also been published.[9,27,28] The sensitivity (Se) of the technique ranges from 44%-100%,[29,30] while its specificity (Sp) is reportedly 36%-100%.[31] The possible causes of such large variations in Se and Sp comprise a lack of consistency in defining the disease and variability in terms of the gold standard and analytical systems used.[32] Furthermore, it must be mentioned that fluorescence-based systems are prone to false-positive readings caused by different factors such as the use of silver amalgam, certain prophylactic pastes, resin composites, or calculus.[33,34]

Electrical impedance systems were designed to assess the extent of the lesion within the enamel,[35-37] and are based on the principle that materials can be characterized by their ability to conduct electricity to one degree or other. Such systems can be used in combination with visual criteria for the diagnosis and monitoring of noncavitated caries.[38] Their use in combination with visual criteria affords better diagnostic performance than visual criteria alone.[39] The system consists of a rechargeable device connected to a sensor that is placed in contact with the examined tooth and a lip clip that closes the circuit. The sensor is a disposable headpiece with a tip ending in a minute series of metal filaments that transmit the current to the tooth in order to obtain the measurements. The sensor is fitted to the upper part of the body of the device, which has a light display that indicates the different lesion degrees based on a luminous color code. A light-emitting diode (LED) screen in the central part of the device indicates the measured values, while the lower part houses the control buttons and an input for connecting the lip clip. The permeability of a tooth increases in the presence of a demineralization process.[40] Such permeability is related to the electrical resistance of the tooth; accordingly, the physical changes present in a carious process can be identified and quantified by measuring this electrical phenomenon. Enamel demineralization is associated with lower electrical impedance compared to sound tissue. Dentin, in turn, has lower impedance than enamel, but its porosity also increases due to dental caries resulting in a decrease in impedance.[41] Systems of this kind should not be used in patients with cardiac pacemakers or for the evaluation of secondary caries or root caries, or to detect the depth of a preparation[16]. These devices come with a screen showing the numerical value (0-100) obtained in the measurement and a color pyramid that lights up (green, yellow, and

red), according to the numerical value obtained.[42] An algorithm, in turn, is used to establish the diagnostic score based on the bioimpedance values mapped against a clinical reference.

The cut-off point is the value used to divide continuous results into categories (typically positive and negative). In this case, positivity is the presence of caries, while negativity corresponds to a sound tooth. Agreement is lacking in the literature on the use of a cut-off point with the different detection systems, and the results of different studies can vary greatly depending on which cut-off point is used.[8,26-28] The differences among the cut-off points found in the literature preclude direct comparison of the different systems.[3,8,28,43]

There is no consensus in the literature regarding the detection capacity of the different diagnostic systems. The Se of the ICDAS II scoring system varies greatly among studies, from 5%-83% for grade D3 (outer half of dentin),[44,45] in the same way as its Sp. The systems based on fluorescence and electrical impedance also show disparate values for different degrees of involvement. Sensitivity ranges from 73%-100%[46,47] and 45%-92%[16,35] for fluorescence and electrical impedance systems, respectively. It is, therefore, essential to carry out more prospective *in vivo* studies involving large sample sizes and using fissurotomy to confirm the true extent of the lesion, as contemplated in our case. The objectives of the present clinical study were to evaluate the ability of impedance spectroscopy to detect occlusal caries, and to compare the technique against fluorescence and visual/tactile examinations. The null hypothesis was that there are no *in vivo* differences in the detection of occlusal caries of the permanent molars and premolars between the fluorescence and electrical impedance systems.

METHODS AND MATERIALS

Study Population

A prospective study was made of 152 patients in the Deparment of Dentistry (Dental Parhology and Therapeutics Unit), University of Valencia between January 1, 2009 and December 31, 2012.

All of the patients were over 18 years of age and presented American Society of Anaesthesiologists' (ASA) classification score I-II. A total of 302 teeth (148 molars and 154 premolars) out of 1758 posterior teeth were analyzed. The teeth were diagnosed with caries amenable to restoration based on the ICDAS system (code 3 or higher), and were also examined with the fluorescence and electrical systems. A bitewing radiograph was subsequently used to confirm lesion extension. The exclusion criteria were teeth with sealants or previous restorations, hypoplasia, or teeth with fluorosis or amelogenesis, in accordance with the recommendations of other authors.[3,8,48]

Clinical Analysis

All the detection procedures were carried out by the same examiner (MM) who was trained and calibrated for all the systems used. The occlusal surfaces were first cleaned using pumice mixed with water and a nylon prophylactic brush (Dentaflux, Madrid, Spain) at low speed. The visual examination was performed under no magnification, as recommended by the ICDAS II code system.[3,15] The tactile examination to check the surface was performed using a TU 17/23 Hu-Friedy exploratory probe (Chicago, IL, USA), moving it over the explored surfaces without applying pressure. The fluorescence system (DD: DIAGNOdent 2095, KaVo, Bibberach, Germany) was used following the recommendations of the manufacturer. Since these were occlusal surfaces, we used the previously calibrated A probe positioned perpendicular to the occlusal surface being examined. In all cases we recorded the highest value obtained.[36] Lastly, the electrical impedance system (IMS: CarieScan PRO, CarieScan Ltd, Dundee, Scotland) was used. The tooth was rehydrated with water from the system syringe for 5 seconds in order to facilitate electrical conductance. We then dried the tooth for another 5 seconds following the recommendations of the manufacturer. The soft tissue clip was placed on the lip of the patient, avoiding contact with any metal restorations present in the mouth. The sensor, in turn, was placed on the occlusal surface of the tooth to be examined, and the "enter" button was pressed to start measuring. Four tones can be heard during the measurement process before the result appears onscreen. The end of measurement is indicated by a long sequence of "beeps" and the classification appearing on the color display.[35] The highest value obtained was recorded. The operating principle is shown in Figure 1.

The true extent of the carious lesions was determined by fissurotomy using round diamond drills measuring 0.5 mm in diameter at high speed (Komet, Gebr Brasseler GmbH & Co, Lemgo, Germany)[8,26] by one operator (MM). The final lesion depth was assessed visually and with the tip of the exploratory probe, evaluating the hardness of the bottom of the fissure. This technique was used as the gold standard in our study. After fissurotomy, bitewing radiographs were obtained to confirm lesion extension, recording the true extent for posterior statistical analysis.[48] The following criteria were used to classify lesion extension: D0 (sound), D1 (outer half of the enamel), D2 (inner half of the enamel), D3 (outer half of dentin), and D4 (inner half of dentin).[16,50]

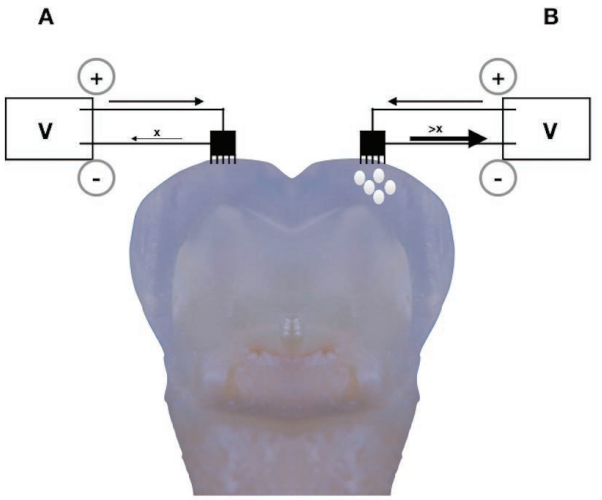

Figure 1. Schematic representation of the electrical impedance system.

Statistical Analysis

A descriptive analysis was made, calculating the Se and Sp values, and the area under the curve (AUC) for each of the systems used. The 95% confidence interval (95% CI) of the AUC was calculated, based on the contrast test AUC=0.5. The inferential analyses included the Mann-Whitney U-test for two independent samples in order to determine whether the distribution of the values of a diagnostic test is homogeneous in two groups. The Kruskal-Wallis test for more than two independent samples was used to determine whether the distribution of the values of a diagnostic test was homogeneous in several groups. The McNemar test was used to compare predictions between the two tests, while the Spearman correlation coefficient was used to estimate the correlation between two at least ordinal variables. The significance level considered was 5% (α=0.05). The statistical power of the study (n=302) was 96% for a two-tailed test, with a level of significance of 5%. The SPSS version 15.0 statistical package (SPSS Inc, Chicago, IL, USA) was used throughout. We used the cut-off points suggested by the manufacturer for both the devices (fluorescence and electrical impedance). In the former system, values from 0 to 14 indicated sound tooth, 15-20 corresponded to D1-D2 lesions, and finally values over 21 corresponded to D3-D4 caries. In the latter system, 0 indicated sound tooth, 1-50 corresponded to D1 caries, 51-90 to D2 lesions, 91-99 to D3 lesions, and 100 indicated D4 caries.

RESULTS

A total of 302 teeth (148 molars and 154 premolars) corresponding to 152 patients with a mean age of 39.1 ± 14.3 years were included. The prevalence of caries in the sample was 72.5% (after fissurotomy).

The fluorescence system provided continuous values. The cut-off points used were those suggested by the manufacturer: D0=17.1% of the sample, D1-D2=10.3%, and D3-D4=72.6% of the lesions. The optimal cut-off point obtained in this study was 23.5. The values referring to diagnostic validity varied according to the real extension of the caries as shown in Table 1.

In relation to the electrical impedance system, the results were classified according to the parameters proposed by the manufacturer: There were no cases with a value of 0, so according to this system there were no sound teeth. Specifically, 46.5%, 20.9%, 0.7%, and 32% of the sample corresponded to D1, D2, D3, and D4 lesions, respectively. The values referring to the diagnostic validity of this system vary according to the degree of extension of the lesion. Other cut-off points (24.5 and 33) were analyzed, and the results are shown in Table 2.

The Se values were D0=27.7%, 96.2%; D1=51.4%, 82,9%; D2=52.3%, 95.2%; D3=94.6%, 98.2%; and D4=95.7%, 100% for ICDAS and DD, respectively. No case was classified as D0, according to the IMS; the Se in this case, therefore, was D1=100%, D2=52.3%, D3=40%, and D4=83.8%. The correlation between the real extension of caries and DD/IMS was 0.911 and 0.904, respectively (p<0.001). The kappa index was 0.50, with 95% CI 0.42-0.67. The Spearman correlation coefficient was 0.911 (r=0.676/ranges), indicating a moderately strong correlation for DD. In the case of IMS, the kappa index of linearly weighted concordance for the complete ordinal classification was 0.59, with 95% CI 0.55-0.63. The Spearman correlation coefficient between the exact value given by the system based on IMS and the actual range of the lesion was r=0.904 (r=0.806/ranges), suggesting a fairly strong correlation. For the AUC estimations, no significant difference was recorded between IMS and DD (p<0.05). However, there were differences between ICDAS and IMS (p<0.001) and between ICDAS and DD (p<0.001). This information is summarized in Table 3. The values corresponding to Se, Sp, AUC, and correlation with fissurotomy of the different methods are presented in Table 4.

DISCUSSION

The present study tested the hypothesis that there are no differences in Se and Sp between the visual and tactile systems and the fluorescence and impedance techniques in detecting occlusal caries of the permanent dentition. Based on the results obtained, the null hypothesis of our study was accepted. In order to establish the true extent of a carious lesion, a biopsy

Table 1: *Results of the Fluorescence System According to Real Extension of the Caries*

	Fluorescence System				Fluorescence System Using Cut-off Value 23.5				
	D0	D1	D2	D3	D4	D0	D1	D2	D3	D4
Sensitivity (Se), %	96.2	82.9	95.2	98.2	100	92.4	74.3	85.7	98.2	100
Specificity (Sp), %	51.2	51.20		49.7		92.7	72.6	57.2	42.8	31.5
Positive (+) predictive value, %	83.5	20.0		62.3		97.0	97.0	84.0	66.0	38.5
Negative (–) predictive value, %	84	84		98.8		82.6	92.4	98.9	100	100
Concordance, %	83.6		61.3			92.4%		—		
Kappa	0.54 (0.42-0.66)		0.50 (0.42-0.57)			0.82 (0.75-0.89)		—		
Correlation fluorescence-real extension	r = 0.911 (p<0.001)					r = 0.911 (p<0.001)				
Correlation range fluorescence-real extension	r = 0.676 (p<0.001)					r =0.755 (p<0.001)				

is needed (in the case of *in vitro* studies) or a fissurotomy must be performed (in the case of *in vivo* studies).[51] It should be noted that not all studies use histology as the gold standard. This is the case of Theocharopoulou and others[52] and Rechmann and others,[53] who used ICDAS as the reference standard. For this reason, not all the published results can be compared. In our *in vivo* study, fissurotomy was taken to be the gold standard for establishing the true extent of the lesions, in concordance with other publications.[8,27,37,54]

Table 2: *Results of the Impedance System According to Real Extension of the Caries*

	Impedance System				Impedance System Using Cut-off Value 24.5					Impedance System using Cut-off Value 33.0				
	D1	D2	D3	D4	D0	D1	D2	D3	D4	D0	D1	D2	D3	D4
Sensitivity (Se), %	100	52.3	40.0	83.8	90.2	44.4	100	100	100	89.8	38.9	100	100	100
Specificity (Sp), %	0	96.6	95.7	93.1	87.8	78.0	56.8	42.4	31.0	96.3	85.6	62.3	46.5	34.0
Positive (+) predictive value, %	41.6	95.2	100	70.5	95.1	95.1	87.3	65.8	39.0	98.5	98.5	91.3	68.9	40.8
Negative (–) predictive value, %	—	82.6	77.5	93.6	78.3	100	100	100	100	78.2	100	100	100	100
Concordance, %	39.4				89.8		—			91.5		—		
Kappa	0.59 (0.55-0.63)				0.76 (0.67-0.84)		—			0.80 (0.73-0.88)		—		
Correlation fluorescence-real extension	r = 0.904 (p<0.001)				r = 0.904 (p<0.001)					r = 0.904 (p<0.001)				
Correlation range impedance-real extension	r = 0.860 (p<0.001)				r = 0.760 (p<0.001)					r = 0.814 (p<0.001)				

Table 3: *Results of the Studied Systems According to Real Extension of the Caries*

	ICDAS	DD	IMS
Sensitivity (Se)	79.0%	92.4%	90.7%
Specificity (Sp)	72.3%	92.7%	87.8%
Area Under the Curve (AUC)	0.756	0.954	0.954
Correlation with fissurotomy	77.1%	89.7%	88.7%

Abbreviations: ICDAS, International Caries Detection and Assessment System; DD, DIAGNOdent; IMS, CarieScan PRO.

We performed visual examination using the ICDAS criteria, in the same way as other authors,[2,8,23] in order to compare the results obtained with those of other publications. In the present study, the Se of ICDAS was seen to increase with caries lesion depth (51.4%-95.6%). Other investigators have recorded similar results. In one study, the Se of ICDAS was found to be 48%-83% in D3 lesions, though in D1 lesions Se performance was found to be 59%-73%.[44] Zaidi and others obtained similar results, with sensitivities of 65% (D1 lesions) and 59% (D3 lesions).[54] Pourhashemi and others[25] found the lowest Se of ICDAS to be 25%, and the highest 61.4% depending on the observer. In another study[45] the Se of IDCAS was 8%-76% (for D1 and D2 lesions) or 5%-78% (for D3 and D4 lesions). The authors reported that clinical performance was not significantly improved when the detection level was moved to the inner enamel layer. Diniz and others,[28] in turn, obtained specificities and sensitivities for enamel and dentin caries versus only dentin caries of 60%-93% and 77%-52%, and similar results were recorded by Jablonski-Momeni and others.[42] There are minor variations between the visual signs associated with each code.[55] Kockanat and Unal[16] reported the highest Se (97-86%) and Sp (96%-93%) at D1 and D3 thresholds of ICDAS. Lower figures were obtained by Singh and others,[56] with a Se of 77.78% and a Sp of 75%. The important differences recorded in the Se of ICDAS can be attributed to the fact that dental caries is a dynamic process in which early lesions undergo demineralization before becoming clinically manifest.

It is difficult to establish comparisons between studies of fluorescence systems, since no consensus regarding the cut-off points can be found in the literature. Each point on a Se-Sp curve corresponds to a cut-off value, and is associated to test Se and Sp. Locating the cut-off point thus requires a compromise between both the parameters. In some cases, Se can be more important than Sp, though in other circumstances the opposite may apply. If there is no preference between Se and Sp, or if both parameters are the same, a good approach would be to maximize both the rates. Different studies have calculated their own cut-off points, seeking to obtain maximum Se and Sp. This is the case of Rodrigues and others, who determined point 30 to be the most suitable cut-off point in terms of Sp.[3] Ghoncheh and others[46] determined three different cut-off points: 8.5, 9.5, and 10.5, with Se values of 73%-92% and Sp values of 57%-82%. In our study, the maximum values for Se and Sp were obtained with a cut-off point of 23.5. Fluorescence system measurements at a single site are reproducible when the device is calibrated according to the instructions of the manufacturer.[57] The classification of a tooth as either presenting caries or being sound or healthy is not conditioned by operator bias, since this is an objective technique

Table 4: *Sensitivity (Se), Specificity (Sp), Area under the Curve (AUC), and Correlation with Fissurotomy of the Different Methods*

		D0	D1	D2	D3	D4
Sensitivity (Se)	ICDAS	27.70%	51.40%	52.30%	94.60%	95.70%
	DD	96.20%	82.90%	95.20%	98.20%	100%
	IMS	—	100%	52.30%	40%	83.80%
Correlation	DD	0.911 (p<0.001)				
	IMS	0.904 (p<0.001)				
Kappa	DD	0.50 95% CI, 0.42-0.67				
	IMS	0.59 95% CI, 0.55-0.63				
Spearman	DD	0.911 (r=0.676/ranges)				
	IMS	0.904 (r=0.806/ranges)				

Abbreviations: ICDAS, International Caries Detection and Assessment System; DD, DIAGNOdent; IMS, CarieScan PRO.

that produces a numerical reading of the inspected site. Nevertheless, the importance of the cut-off point and the influence according to some authors of stains in fissures and cracks are clear. Such stains could be a source of high false-positive rates obtained with light fluorescence, with a consequent decrease in the Sp values recorded.[24,58] The high incidence of false-positive results produced by laser fluorescence devices not only reduces the Sp values but is also partially responsible for higher Se values.

The Se obtained with the fluorescence system lies within a narrow range (79%-100%).[51] However, the Sp reported for this system in the literature is lower than that obtained in our study, where the range was seen to be 53%-72%.[3,59,60] When caries affects dentin, the Se of the technique is close to 100%. However, in the case of D1 and D2 lesions, the Se values are lower. The Se of the system was seen to increase with the severity of the lesion (82.9% in D1 lesions versus 100% in D4 lesions). Our results are consistent with those of other studies.[26,43] Some authors consider that fluorescence readings reflect changes in the organic material rather than in the inorganic content of the teeth. Oral bacterial metabolites affect the signal, and an increase in fluorescence is due more to changes in the organic content of carious lesions than to mineral disintegration.[61,62]

With the fluorescence technique, the differences among the cut-off points found in the literature preclude direct comparison of the systems studied. We used the cut-off points proposed by the manufacturer, but other cut-off points have also been studied, and the findings have been presented in the Results section. Teo and others[34] included different cut-off points. The D1 cut-off point (21) was a matter of concern, even when the second chosen cut-off point (51) represented deeper lesion extension into the inner-third of the enamel layer. In the same study, with the mentioned cut-off point of 21, the Sp was 81.7% and increased to 100% with the other two cut-off points (51 and 91, proposed by the manufacturer, as in our case). In the last two cases, Se decreased to 71.6% and 45%, respectively. The overall values in the study by Teo and others were 95% for Se and 44% for Sp. The high Se obtained by these authors suggests that the system is able to detect correctly (enamel and dentin) but is hampered by low Sp that does not allow it to distinguish between caries and healthy dental tissue. In D2 lesions and deeper lesions, the results clearly improved. This system yielded the highest Cohen's kappa coefficients in the study published by Katge.[37] On the other hand, Kockanat and Unal, in primary molars, reported higher Se for D1 lesions (89%-92%) than for D3 lesions (47%-73%).[16] This difference can be explained because more than lesion depth is responsible for the result or value obtained by the system. In an *in vitro* study of 10 posterior teeth subjected to electrical impedance measurements and microcomputed tomography scans, the analysis of the images revealed a complex morphology of occlusal caries in terms of density. The authors concluded that lesion morphology is an important factor to be taken into account in assessing the capacity of electrical impedance spectroscopy to detect caries or conduct follow-up of carious lesions over time.[63] This may explain the high values obtained in our study, even though lesion depth was smaller than in other cases where the numerical reading obtained was lower. It is also important to note that the histology of a tooth changes with advancing age—with significant differences in measurements between young and mature teeth. The formation of peritubular dentin over time causes the tubular lumen to gradually narrow and even become obliterated.[40] A study in which electrical impedance measurements were obtained from 99 healthy first molars every 6 months found impedance to increase in the posteruptive period.[64] For this reason, and in the same way as there are differences between the deciduous and permanent teeth, the values obtained with this system cannot be generalized to all ages when establishing a treatment plan.[35]

Some studies have found electrical impedance systems to be more sensitive in detecting occlusal caries than visual, tactile, or radiographic techniques. Katge and others[37] found the Se of this system to be 97%, versus 93% in the case of ICDAS. However, Sp was lower (82%) for electrical impedance, though other authors claim the opposite.[16,48,65] The lower Se values reported by some investigators underscores the importance of this system in monitoring lesions confined to dentin and healthy teeth. Jablonski-Momeni,[42] in a study of 292 teeth, recorded high *in vivo* reproducibility but only moderate correlation to the findings of the visual system. The authors considered the system to be useful for monitoring caries not subjected to invasive treatment but questioned its validness in detecting lesions limited to the enamel—visual examination being considered more useful in this case. This differs from our own findings, in which electrical impedance yielded better results than ICDAS in D2 caries (Se 100% versus 52.3%). The lack of a gold standard in the above-mentioned study for validating the results may be the cause of this difference. Also, Jablonski-Momeni and others conducted their study in adults over a broad age range (18-68.5 years), and hence the results may differ from those obtained in our study.

Identifying the initial stages of demineralization is essential in order to allow noninvasive therapies such

as fluoride therapy. According to the clinical practice guidelines referred to nonrestorative treatments for carious lesions of the American Dental Association,[66] clinicians should prioritize the use of sealants plus 5% NaF varnish applied every 3-6 months, or sealants alone over 5% NaF varnish alone, or 1.23% APF gel applied with the same frequency. As an application at home, 0.2% NaF mouth rinses once per week are strongly recommended.

With regard to the limitations of our study, it must be mentioned that due to ethical concerns, the examined teeth were diagnosed with caries amenable to be restored based on the ICDAS system. Furthermore, the study design did not allow us to determine whether any of the evaluated detection methods are useful for detecting truly incipient caries lesions.

CONCLUSIONS

Clinically, the electrical system on its own is not useful for differentiating between sound teeth and truly incipient caries lesions. The fluorescence or electrical systems are recommended with the ICDAS to detect carious lesions in their early stages.

Regulatory Statement

The study and experimental protocol were reviewed and approved by the Institutional Review Board of the University of Valencia (IRB00010108) and were conducted in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all the participants included in the study.

Conflict of interest

The authors declare that they have no proprietary, financial, or other personal interests of any kind in relation to any product, service, and/or company cited in this article.

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NaOCl Application after Acid Etching and Retention of Cervical Restorations: A 3-Year Randomized Clinical Trial

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Clinical Relevance

The cavity pretreatment with NaOCl solution seems to yield no clinical advantages for restoring NCCLs using composite resins.

SUMMARY

This study evaluated the retention of composite resin restorations in noncarious cervical lesions (NCCLs) performed with or without pretreatment with 10% NaOCl solution (deproteinization). A randomized, controlled, split-mouth, double-blinded trial was carried out. Thirty patients with at least two NCCLs were included in the study. The NCCLs were randomly allocated into two treatment groups: control (acid etching with 37% phosphoric acid + placebo solution + Adper Single Bond 2/3M Oral Care + Filtek Z350/3M Oral Care) or experimental group (acid etching with 37% phosphoric acid + 10% NaOCl solution + Adper Single Bond 2 + Filtek Z350). A calibrated examiner evaluated the restorations at baseline (1 week) and recalls (6, 12, 24, and 36 months) using the FDI criteria. The primary outcome evaluated was retention of the restorations. Data were analyzed by the Kaplan-Meier method and the log-rank test (α=0.05). After 3 years, 64 restorations were evaluated in 23 patients. The annual failure rate was 9% for the control group and 17.8% for the

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experimental group (deproteinization technique). Considering the failures and their distribution among the characteristics of the patients and NCCLs, no statistically significant differences were observed for the control and experimental treatment groups (p=0.077) or the number of teeth in the mouth (p=0.320). Restorations in the mandible (p=0.039) and premolars (p=0.013) showed significantly lower clinical survival rates. The deproteinization pretreatment with a 10% NaOCl solution did not promote additional retention of restorations in NCCLs. (clinicaltrials.gov: NCT03086720)

INTRODUCTION

Effective and stable dentin bonding is fundamental for the long-term durability of composite resin restorations, particularly when little or no marginal enamel is available.[1] In challenging restorative situations such as noncarious cervical lesions (NCCLs), loss of restoration retention is a common clinical problem.[2] Clinical studies on NCCLs restored with composite resin without mechanical retention constitute a good model for evaluating the clinical performance of adhesive restorations, since in these situations the retention of restorations relies on the bonding agent and adhesive procedure.[3-5] The prognosis of cervical restorations may also be affected by other factors, including clinical characteristics of the cervical lesion and patient-related aspects.[2,6,7]

Laboratory and clinical studies have shown that the application of phosphoric acid is not the best approach to achieve bonding to dentin,[8] especially in patients with symptomatic NCCLs. Acid etching may generate demineralized areas beneath the hybrid layer, leaving exposed collagen fibrils not impregnated by the adhesive.[9] These areas may allow access to the entry of water into the adhesive layer and cause postoperative sensitivity.[5] The exposed collagen fibrils also may undergo hydrolysis, interfering with the stability of the bonded assembly.[8] Some strategies to potentially improve dentin bonding have been proposed, especially for etch-and-rinse adhesive protocols. Inhibition of enzymatic biodegradation has been suggested; however, apart from the satisfactory laboratory evidence,[10,11] the clinical data about the contribution of this enzymatic activity to bond degradation is still unclear.[12,13]

Removal of the dentin collagen mesh exposed by acid etching could also be an optional strategy for improving bonding to dentin.[14] When the collagen mesh is removed, the adhesive interlocks with the etched hydroxyapatite without the formation of a collagen-adhesive hybrid layer. The collagen could be removed by using a deproteinization protocol, usually involving the application of sodium hypochlorite (NaOCl)[15-17]—a nonspecific proteolytic agent capable of dissolving collagen. This ability of NaOCl is influenced by the time of exposure, concentration of active chlorine, and superoxide radicals in the solution.[18,19] Many *in vitro* studies have reported stability of dentin bonding after deproteinization protocols.[18-21] Although some positive results have been reported for the deproteinization technique, other *in vitro* studies have shown that dentin pretreatment with NaOCl did not lead to better bonding results compared with the conventional adhesive technique.[22,23] Clinical investigations on the performance of restorations performed with the deproteinization technique are scarce.[14,24] Both a 2-year clinical pilot study and a 5-year clinical study concluded that NaOCl pretreatment in the restoration of NCCLs was encouraging, since it did not affect the clinical performance of NCCL restorations.[14,24] Studies have suggested that the type of adhesive system might interfere with the performance of the technique.[14] Further clinical studies are still necessary to provide results that will enable conclusions to be drawn about the *in vivo* applicability of deproteinization bonding protocols, especially considering longer follow-ups.

The deproteinization technique with NaOCl could be a significant procedure for improving the adhesive stability and restoration retention, whereas it could be yet another clinical procedure that only increases treatment time. This randomized controlled clinical trial aimed to evaluate the retention of composite resin restorations in NCCLs performed with or without pretreatment of the acid-etched dentin with a 10% NaOCl solution. The null hypothesis tested was that the deproteinization technique does not influence the failure rates after a 3-year follow-up period.

METHODS AND MATERIALS

This study is reported in accordance with the guidelines of the Consolidated Standards of Reporting Trials (CONSORT).[25]

Study Design

This study was a randomized, controlled, split-mouth, and double-blinded trial with a 3-year period of follow-up. The control group used a placebo solution, and the experimental group used 10% NaOCl solution as pretreatment after dentin acid etching and before application of the adhesive agent. In this split-mouth study, the patient and outcome evaluator were blind to the control and experimental groups. However, it was not possible to blind the operators because of the

odor of the NaOCl solution. Restorations were placed between 2011 and 2012 by 10 different operators, who were undergraduate students of a dental school, in the last year of their course, working under the supervision of an experienced clinician and researcher (AFM).

Operator Training

Theoretical and practical training minimized variation among the operators. The undergraduate students received a manual containing instructions for the application of all materials and protocols of the clinical procedures. All operators underwent preclinical demonstrative training before the study began. Afterwards, they performed cervical restorations in a number of teeth of volunteer patients, corresponding to at least 10% of the total NCCLs sample size of the study. These restorations and patients were not included in the study sample; they were treated as a part of the regular undergraduate training of the students.

Sample Size

Taking into account a rate of retention of 87% after 36 months of placing the composite resin restorations in NCCLs, and the lack of difference with the control group,[26] the sample size calculation for an equivalence trial was based on a limit of 25% difference in retention rates between the groups, at a significance level of 5% and power of 80%. Considering the loss of subjects during the follow-up period, 10% were added; thus, the final sample size was composed of 30 patients, in a study with a split-mouth design.

Recruitment and Selection of Patients

Information about the study was disseminated by distributing posters and pamphlets at the dental school. All patients who were referred to the school clinics for treatment were evaluated and treated accordingly, when necessary. The inclusion criteria for participation in the study were adults, capable of understanding the informed consent form, with at least two NCCLs in incisors, canines, or premolars, with more than 20 teeth in the mouth, and with good periodontal health. Excluded from the study were smokers, bruxers, patients with severe systemic diseases, patients undergoing orthodontic treatment, teeth without antagonists or with wear facets covering more than 50% of the incisal and/or occlusal surfaces, presence of caries or restorations in the area to be treated, visible plaque index or gingival bleeding index higher than 20%, probing depth and clinical attachment loss greater than 4 mm with bleeding on probing, and patients lacking interest in returning for follow-ups or who refused to participate.

Patients who fulfilled the eligibility criteria received an informative letter about the purpose of the study, and a term of free and informed consent [form] to be signed, confirming their voluntary interest in participating. All participants were submitted to a detailed initial clinical examination, including several criteria regarding the classification of NCCLs. The criteria for evaluating NCCLs consisted of the following parameters: cervical lesion shape ("U" or "V"), length and height of the lesion (in mm), relation of the cervical margin with the gingiva (supragingival, gingival level, or subgingival), presence of wear facets, presence and degree of dentin sclerosis (when present), dentin sensitivity, and pulp vitality.

Randomization and Blinding Procedures

Randomization was performed using MS Excel computer software by a researcher (TPC) who was not directly involved in the study at the time. A random table was used to allocate the NCCLs to each study group. The treatments (control and experimental) were allocated, considering the tooth group (incisors, canines, and premolars), for which the first tooth to be restored was randomly allocated to one treatment. In contrast, the next tooth from the same tooth group was automatically assigned to the other treatment, according to the split-mouth design. Thus, in both the groups, each patient received the same number of restorations. Ten operators (undergraduate students) performed a similar number of restorations. Each operator performed the same number of restorations in both the groups. Individual opaque sealed envelopes were used to conceal the randomization sequence, which was coded as Treatment A or Treatment B. The same clinical sequence and identical solution bottles were used for both the groups.

Clinical Procedures

Before the adhesive procedures, prophylaxis of the tooth was performed with a rubber cup and pumice-water paste. No cavity preparation or beveling of the cavosurface margin was performed. The composite resin shade was selected using the Vitapan Classical shade guide (Vita Zahnfabrik, Bad Säckingen, Germany). When necessary, local anesthesia was used. Isolation of the operative field was performed using a labial retractor, gingival retraction cord #0000 (Ultrapak Cord; Ultradent, South Jordan, UT, USA), cotton rolls, and a suction device. The dentin was etched with 37% phosphoric acid gel (Adper Scotchbond Etchant, 3M Oral Care, St. Paul, MN, USA) for 15 seconds, rinsed with air-water spray for 30 seconds, and the excess moisture was removed from dentin with absorbent

paper. For the Experimental Group, a 10% NaOCl solution (Uso Indicado Pharmacy, Pelotas, RS, Brazil) was applied with a disposable pharmaceutical syringe and remained in contact with the dentin surface for 60 seconds. The surface was rinsed with air-water spray for 30 seconds to remove all the excess NaOCl. For the control group, the same sequence was followed but using a placebo solution (water).

For both groups, the application of a two-step adhesive system (Adper Single Bond 2, 3M Oral Care) and restorative technique using a nanofilled composite resin (Filtek Z350, 3M Oral Care) were performed in accordance with the manufacturer's instructions. Each composite resin increment was cured for 20 seconds with a light-emitting diode (LED) light-curing unit (Radii-Call; SDI, Bayswater, VI, Australia) with an irradiance of 800 mW/cm^2. All the restorations were finished with #12 scalpel blades, fine and ultrafine diamond burs (KG Sorensen, Barueri, SP, Brazil) under water cooling to remove excess material and/or improve the shape and contour of the restoration. Polishing was performed with silicone tips, alumina abrasive discs (Sof-Lex Pop-On; 3M Oral Care), felt disks, and diamond polishing paste (Prisma Gloss; Dentsply Caulk, Milford, DE, USA).

Clinical Assessment

A previously trained, calibrated, and blinded examiner (MSC) who has worked as an examiner in other clinical studies carried out the clinical evaluations of restorations at baseline (1 week) and follow-up time intervals (6, 12, 24, and 36 months). Training and calibration procedures used a web-based training and calibration tool (www.ecalib.info) and clinical setting evaluations. Thirty NCCL restorations (4 patients) were reexamined 15 days later, to provide clinical intraexaminer calibration. A preevaluation intraexaminer agreement above 90% was obtained. The examiner used the criteria approved by the FDI World Dental Federation.[27] The primary outcome was retention of the restoration, and the complete loss of a restoration was considered a failure. Secondary endpoints included marginal staining, postoperative sensitivity, surface gloss, translucency, color, fracture, anatomical shape, preservation of vitality, and integrity of teeth. Each criterion was expressed in five scores—three scores for clinically acceptable restorations and two scores for unacceptable restorations (in need of repair or replacement).

Recalls

Patients were contacted by telephone and asked to attend scheduled appointments for reevaluations after 6, 12, 24, and 36 months. In case of unsuccessful telephone contact, a letter was sent to the residential address provided in the clinical record, or home visits were made by the researchers and evaluators involved in the recalls (MSC and MF). In these follow-up exams, the restorations were evaluated according to the FDI criteria, and photographic records were taken.

Statistical Analysis

Statistical analysis was performed using Stata 14.2 software (Stata Corp LP, College Station, TX, USA). Descriptive analysis was used for the variables of interest. Differences between frequencies were assessed by Fisher Exact test. Distribution and frequency data for the presence/absence of failures were measured with the Chi-square test. Survival analysis was performed using the Kaplan-Meier method, followed by the log-rank test. Unadjusted Cox regression models with shared frailty were used to verify the association between the treatments and risk of failure over time, estimating the hazard ratios (HR) and 95% confidence intervals (CI). In all analyses, α=0.05 was considered.

RESULTS

During enrollment from September 2011 to August 2012, 61 patients were assessed for eligibility: 31 did not fulfill the inclusion criteria or did not want to participate, while 30 patients (17 men, 13 women), with an average age of 49 years were included in this study, for placement of a total of 100 NCCL composite resin restorations. Details of the recruitment procedures, exclusions, losses, and the number of participants at each recall of the trial are disclosed in the study flowchart (Figure 1). After 36 months of follow-up, the average lifetime of the restorations was 2.9 years.

In Table 1, it is possible to observe the characteristics of the subjects evaluated at the last recall (36 months). Table 2 shows the features of the NCCLs. The majority of patients had between 20 and 24 teeth in the mouth, and 63.3% of the patients reported the consumption of acidic foods and/or sour drinks. However, within the sample studied, a higher number of lesions presented in patients who did not report an acidic diet (Table 3), but the difference was not statistically significant. The majority of lesions had a V wedge shape (60%), 48% had a depth below 1 mm, and 49% had a height between 1 and 3 mm. NCCLs were predominantly present in premolars (58%).

At 36 months, the annual failure rates (AFRs) were 9% for the control group and 17.8% for the experimental group (NaOCl/deproteinization technique). Kaplan-Meier survival curves for different study variables are presented in Figure 2. When observing the failures

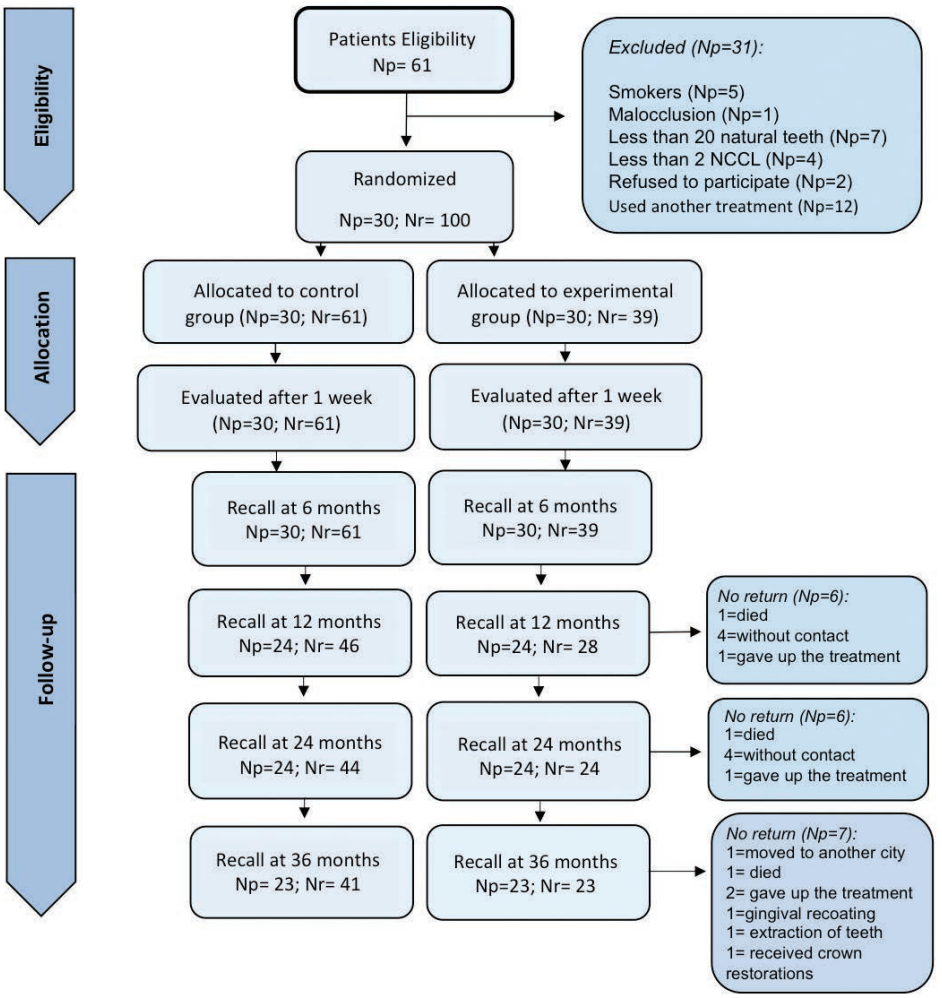

Figure 1. *Flowchart showing participants' enrollment. Abbreviations: Np, number of patients; Nr, number of restorations.*

Table 1: *Characteristics of the Subjects Included in this Study (n=30)*

Characteristic	Subdivision	N (%)
Number of teeth	20-24	17 (56.7%)
	25+	13 (43.3%)
Income	Up to 2 BMW	16 (53.3%)
	>2 BMW	14 (46.7%)
Educational level, years	Up to 8	9 (29%)
	9-11	16 (51.6%)
	>11	6 (19.4%)
Age, years	20-40	5 (15.6%)
	41-60	23 (71.9%)
	>60	4 (12.5%)
Acidic diet	Yes	19 (63.3%)
	No	11 (36.7%)

Abbreviations: BMW, Brazilian minimum monthly wage.

and their distribution among the characteristics of the patients and NCCLs, no significant differences were observed between the control and experimental groups (p=0.077, Figure 2A), or the number of teeth in the mouth (p=0.320, Figure 2C). Mandibular restorations (p=0.039, Figure 2B) and restorations in premolars (p=0.013, Figure 2D) had significantly lower clinical survival. The Cox regression analysis confirmed these results regarding the significance of the associations (Table 4). Regarding the type of treatment, there were 40% more failures in the Experimental Group (NaOCl) when compared with the control Group, but the association was not significant (p=0.075).

When the data were submitted to the Chi-square test (Table 3) to measure the quantity and percentage of data according to the presence of failure or not, no significant difference in the failure rates between the experimental and the control groups was observed (p=0.054). Table 3 presents the number and percentage

Table 2: *Characteristics of the Noncarious Cervical Lesions Restored in the Study (n=100)*

Characteristic	Subdivision	Control Group, n (%)	Experimental Group, n (%)
Tooth type	Incisor	15 (71.4%)	6 (28.6%)
	Canine	15 (71.4%)	6 (28.6%)
	Premolar	31 (53.5%)	27 (46.5%)
Dental arch	Maxilla	34 (69.4%)	15 (30.6%)
	Mandible	27 (52.9%)	24 (47%)
Lesion depth	<1 mm	28 (58.3%)	20 (41.7%)
	1-3 mm	29 (63%)	17 (37%)
	3-4 mm	1 (50%)	1 (50%)
	>4 mm	2 (100%)	0 (0%)
Lesion height	<1 mm	3 (42.9%)	4 (57.1%)
	1-3 mm	30 (61.2%)	19 (38.8%)
	3-4 mm	20 (62.5%)	12 (37.5%)
	>4 mm	4 (66.7%)	2 (33.3%)
Cavity shape	U saucer shape	26 (65%)	14 (35%)
	V wedge shape	35 (58.3%)	25 (41.7%)

of restorations for each variable studied. The variables studied, such as the patient-related variables (income, educational level, and age) and local variables (acid ingestion, depth, and height of NCCLs, cavity shape, degree of sclerosis, and restoration margin) showed no statistically significant effect on the retention of restorations. The type of the tooth and its position in the maxillary or mandibular arch seem to influence the retention of the restoration, showing that restorations in the mandible and in premolars suffered more retention failures.

With regard to tooth sensitivity, obtained by means of a questionnaire answered by the patients before and after the restorations were carried out, 37.5% of patients reported that they never had tooth sensitivity, 56.3% reported improvement in tooth sensitivity after restoration, and 6.3% of patients reported that the restored teeth remained sensitive in all of the follow-up time intervals. No patient in the NaOCl group reported sensitivity after restoration placement, while five patients in the control group (placebo) reported that the teeth remained sensitive. However, there was no difference in tooth sensitivity between the groups (p=0.270). After the 36-month follow-up period, the majority of restorations for both the groups (control and experimental) had an FDI score 1, which represents a clinically excellent result (Table 5). This finding demonstrated that the restorations remained satisfactory over time, irrespective of the treatment.

DISCUSSION

The main finding of this study was that the application of a deproteinization technique using 10% NaOCl solution after dentin acid-etching to remove the exposed collagen mesh had no positive impact on the retention of composite resin restorations in NCCLs after 3 years of clinical service. The majority of NCCL restorations remained satisfactory over time, irrespective of the treatment. The AFRs were 9% for the control group (conventional bonding technique) and 17.8% for the Experimental Group (NaOCl/deproteinization technique) after 3 years. Thus, the null hypothesis tested was accepted.

The results reported for the deproteinization technique in this clinical study were in disagreement with the positive effects reported in laboratory experiments.[18-21] Theoretically, the deproteinization technique would be able to remove the collagen mesh exposed by acid etching and make the dentin bonding assembly less prone to hydrolytic degradation or enzymatic action. However, it was reported that NaOCl could not wholly remove the collagen fibrils within clinically relevant application times[28]; thus, the bonded interface was not entirely free of collagen. Furthermore, the mismatch between acid-etched and adhesive-impregnated dentin areas may still occur in the deproteinization technique,[17] interfering with long-term bonding performance.

Results of clinical studies on the efficacy of the deproteinization technique on restoration retention are scarce, especially considering longer follow-ups.

Table 3: *Number and Percentage of Retention for Each Variable Studied (n=100)*

Variable		Survival (Retention)	Failure (Loss of Retention)	*p*-Value
Income	Up to 2 BMW	38 (74.5%)	13 (25.5%)	0.399
	>2 BMW	30 (66.7%)	15 (33.3%)	
Treatment groups	Control	47 (77%)	14 (23%)	0.054
	Experimental	23 (59%)	16 (41%)	
Educational level, years	Up to 8	21 (87.5%)	3 (12.5%)	0.187
	9-11	34 (65.4%)	18 (34.6%)	
	>11	11 (64.7%)	6 (35.3%)	
Age, years	20-40	5 (45.5%)	6 (54.5%)	0.083
	41-60	47 (70%)	21 (30%)	
	>60	16 (84.2%)	3 (15.8%)	
Acidic diet	Yes	29 (72.5%)	11 (27.5%)	0.625
	No	38 (67.9%)	18 (32.1%)	
Number of teeth	20-24	42 (62.7%)	25 (37.3%)	0.051
	25+	24 (82.8%)	5 (17.2%)	
Lesion depth	<1 mm	32 (66.7%)	16 (33.4%)	0.110
	1-3 mm	35 (76.1%)	11 (23.9%)	
	3-4 mm	0 (0%)	2 (100%)	
	>4 mm	1 (50%)	1 (50%)	
Lesion height	<1 mm	5 (71.4%)	2 (28.6%)	0.104
	1-3 mm	40 (81.6%)	9 (18.4%)	
	3-4 mm	18 (56.3%)	14 (43.7%)	
	>4 mm	4 (66.7%)	2 (33.3%)	
Tooth type	Incisor	20 (95.2%)	1 (4.8%)	0.011
	Canine	15 (71.4%)	6 (28.6%)	
	Premolar	35 (60.3%)	23 (39.7%)	
Dental arch	Maxilla	40 (81.6%)	9 (18.4%)	0.013
	Mandible	30 (58.2%)	21 (41.2%)	
Cavity shape	U saucer shape	27 (67.5%)	13 (32.5%)	0.656
	V wedge shape	43 (71.7%)	17 (28.3%)	
Dentin sclerosis	Absent	36 (76.5%)	13 (26.5%)	0.193
	Slight	17 (58.6%)	12 (41.4%)	
	Moderate	14 (82.4%)	3 (17.6%)	
	Severe	1 (33.3%)	2 (66.7%)	
Restoration margin	Supragingival	14 (53.8%)	12 (46.2%)	0.681
	Gingival level	45 (63.4%)	26 (36.6%)	
	Subgingival	2 (66.7%)	1 (33.3%)	

Abbreviations: BMW, Brazilian minimum monthly wage.

Previous studies evaluated the composite restorations of NCCLs with or without collagen removal with 10% sodium hypochlorite NaOCl[14,24]; however, they did not explore some critical local- and patient-related factors that could influence the prognosis and restoration survival rates, such as the income and educational level, type of teeth, and position of the tooth in the dental arch.[2,6,7,30]

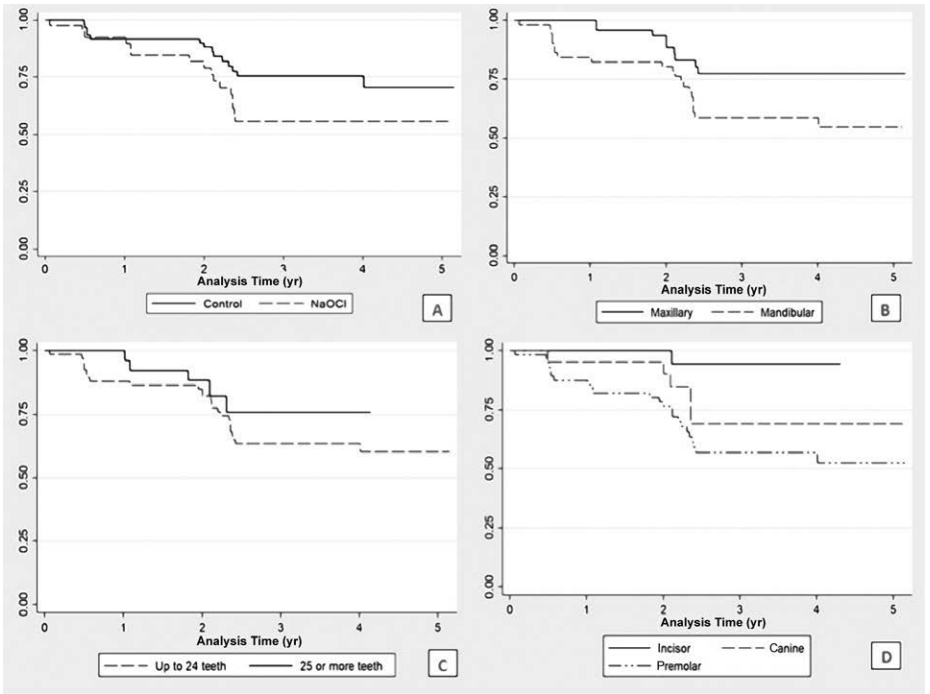

Figure 2. *Kaplan-Meier survival curves for A: type of treatment (experimental or control,* p=*0.077); B: dental arch (maxillary or mandibular,* p=*0.039); C: number of teeth in mouth (20-24 or -25 or more,* p=*0.320); and D: tooth type (incisor, canine, or premolar,* p=*0.013).*

The present findings corroborated those of previous studies showing that the adhesive procedure with a deproteinization technique, which is an extra step during the adhesive protocol, had no positive impact on the clinical service of composite restorations.[14,24] This finding indicated that there seemed to be no reason to continue testing this deproteinization technique, both *in vitro* and *in vivo*. In dental materials research, it is somewhat common to encounter contradictory findings between *in vitro* and *in vivo* studies. For instance, there has been discussion about the different mechanical results between dental restorative composite resins and ceramics observed in laboratory experiments having translated into minor effects in the clinical scenario,[29-31] which appeared to be the case here. Preclinical studies are of utmost importance in the health sciences; however, the conditions are usually far more controlled than in the clinical situation.

Table 4: *Hazard Ratios (HR) and 95% Confidence Intervals (CI) for Failure of the NCCLs Restorations According to Clinical Variablesa*

Variables	HR (95% CI)	*p*-Value
Treatment		0.075
Control - placebo	1.00	
Experimental - NaOCl	1.41 (0.97-2.05)	
Tooth type		0.046
Incisor	1.00	
Canine	5.81 (0.62-54.79)	
Premolar	10.83 (1.35-87.10)	
Position		0.027
Maxilla	1.00	
Mandible	2.68 (1.12-6.45)	
Number of teeth		0.337
20-24	1.00	
25 or more	0.53 (0.15-1.94)	

Abbreviations: NCCL, noncarious cervical lesions.
[a] Cox regression analysis with a shared frailty factor.

The contemporary literature has pointed out that NCCLs are probably caused by a combination of clinical factors,[32-34] which often favor a less-accurate diagnosis by dentists.[35] The shape and size of the lesions will depend on specific clinical factors. In this study, 58% of NCCLs were present in premolars, which were the teeth in which significantly more restoration failures occurred, and 60% of the total sample had a V-wedge shape. Reports on the biomechanical behavior of NCCLs have indicated that V-shaped lesions may show higher stress concentration, especially at the apex of the lesion,[32] in comparison with U-shaped lesions.[33] One study[33] showed that stresses in the depth of the lesions were distributed over a wider area for U-shaped lesions, whereas, for the V-shaped lesions, the stresses were concentrated over a narrow area. Taking all these data into account, it may be suggested that the majority of the sample in the present study was composed of lesions with poorer prognosis in terms of biomechanical behavior. However, it has also been

Table 5: *Comparison Between the Groups Considering the Restorations Remaining After 36 Months, According to the FDI Criteria Compared by Fisher's Exact Test*

General Criteria	Specific Criteria	Control Group	Experimental Group	*p*-Value
		FDI Scores (1/2/3/4/5)[a]	FDI Scores (1/2/3/4/5)[a]	
Esthetics properties	Superficial brightness	17/16/6/0/0	8/7/4/0/0	0.869
	Surface staining	20/16/3/0/0	10/5/4/0/0	0.297
	Marginal staining	5/20/13/1/0	2/12/4/1/0	0.697
	Translucency and color stability	9/16/13/1/0	5/8/6/0/0	1.000
	Anatomic form	21/13/4/1/0	8/11/0/0/0	0.185
Functional properties	Fracture	37/1/1/0/0	19/0/0/0/0	1.000
	Retention	37/1/1/2/0	19/0/0/4/0	0.281
	Marginal adaptation	14/21/4/0/0	3/16/0/0/0	0.072
	Patient perception	38/1/0/0/0	19/0/0/0/0	1.000
Biological properties	Postoperative sensitivity	35/1/3/0/0	18/1/0/0/0	0.591
	Tooth vitality	39/0/0/0/0	19/0/0/0/0	1.000

[a] *Numbers separated by slash represent the number of evaluated restorations rated for each score, according to the FDI criteria: 1. Clinically excellent; 2. Clinically good; 3. Clinically sufficient/satisfactory; 4. Clinically unsatisfactory; 5. Clinically poor.*

shown that the presence of adhesive restorations may help with generating a biomechanical behavior similar to that of sound teeth, thereby overcoming the concerns of stress concentration.[36,37] One study[38] showed that restored lesions subjected to loading at the buccal cusp tended to concentrate stresses at the gingival restorative interface. In addition, the dimension of the lesion and periodontal support status may have a significant impact on the stress distribution.[38]

A previous study evaluating the influence of tooth isolation techniques (rubber dam vs cotton roll isolation) on retention of the noncarious cervical lesion and on the occurrence/progression of gingival recession showed that the rubber dam isolation did not promote further restoration retention of NCCLs and is a risk factor for occurrence/progression of gingival recession after 5-year follow-up.[39] In the present study, the restorations were performed under cotton roll isolation. Moreover, the cotton roll isolation was effectively performed using a labial retractor, gingival retraction cord, an effective suction device, and the operator performed all clinical procedures with the help of a dental assistant. Nevertheless, moisture control may be less controlled in the mandible, in spite of all the efforts made to provide moisture-free areas for bonding. More restoration failures were observed in the mandible compared with the maxilla. A possible reason for this result may be the fact that the restorations were not placed under rubber dam isolation. The clinical relevance of this finding relies on the prognosis for restoration longevity in mandibular teeth, particularly premolars. Nevertheless, these negative results pointed out that the use of NaOCl would not only increase restoration cost and chair time but would also not provide the patient with any real clinical effect. Another clinical finding was that postoperative sensitivity was not common after restoration placement. Interestingly, only teeth in the control group remained sensitive, whereas the deproteinization technique was able to eliminate tooth sensitivity; this finding was not significant. However, if this feasible positive influence on postoperative sensitivity were to be confirmed in other studies, there are different ways to manage tooth sensitivity; and the use of NaOCl should not be encouraged for this purpose.

CONCLUSIONS

Application of 10% NaOCl solution as a deproteinization treatment after dentin acid etching and before application of a two-step adhesive in Class V NCCLs did not lead to any significant improvement in retention. The deproteinization technique did not improve any other restoration clinical parameter when compared with the conventional bonding technique for composite resins, after 3 years of clinical service. The deproteinization pretreatment seemed to yield no clinical advantages for restoring cervical lesions.

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Regulatory Statement

The Local Research Ethics Committee approved this research project (Protocol #210/2011). The study was registered in clinicaltrials.gov (NCT03086720).

Conflict of Interest

The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript. 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. The authors alone are responsible for the content and writing of this paper.

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Repair of Dental Restorations: A 10-year Retrospective Analysis of Clinical Data

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Clinical Relevance

The repair of dental restorations is one of the pillars of minimally invasive dentistry. It is clinically relevant to show that the number of repairs has increased over the years in a dental school as this may have an impact on the clinical decisions of professionals of future generations.

SUMMARY

Objectives: This study collected and analyzed clinical data regarding the repair of dental restorations in patients treated in the clinics of a dental school over 10 years.

Methods and Materials: Data related to repair procedures for permanent tooth restorations were extracted from the digital dental records system and filtered according to year (January 1, 2008, to December 31, 2017), age (<30, 30-60, >60), tooth group, and dental surfaces. Data were analyzed with descriptive statistics in terms of the absolute and relative frequency, and chi-square tests (95% confidence) were used to compare the frequency of repairs between years, age, tooth, and dental surfaces.

Results: A total of 48,915 dental records were accessed by searching for general restorative procedures, of which 1,408 were repairs of dental restorations on permanent teeth. The number of repairs per year increased over the period assessed, and there was a significant increase in the years 2016 and 2017. Individuals aged between 30 and 60 years received the largest number of repairs, with

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significantly more repairs than the other groups. Regarding the tooth group and surface, the canines and the incisal and lingual surfaces received the least number of repairs.

Conclusions: The number of repairs increased over the study period. When comparing frequencies between groups, those belonging to the 30- to 60-years of age group received more repairs; the least repaired surfaces were the lingual and the incisal.

INTRODUCTION

The decision whether to maintain, repair, or replace dental restorations is a topic of interest.[1,2] In this context, the idea behind minimally invasive dentistry is that by removing only the failing portion of a restoration, it is possible to achieve good clinically restorative performance through a restorative repair.[3-5] When repairing a restoration, compared to doing a complete replacement, advantages include reduced material costs[6] and a shorter clinical time required to perform the procedure, which may reduce the patient's anxiety.[2] In addition, the decision to repair a restoration is a more conservative option compared to the replacement option.[7] The intervention strategy used in minimally invasive dentistry involves the detection of early caries lesions, caries risk assessment, clinical decision-making, and personalized care planning.[8]

Previous studies have suggested that the clinical application of minimally invasive dentistry may be beneficial. Van de Sande and others[9] collected 15 years of data from patient files of a private dental practice, investigating whether restorative repair in anterior teeth was a good clinical alternative to replacement. The authors concluded that a composite resin repair might be a good option for anterior teeth, as it could increase the survival of their restorations over time. In another study[10] that investigated whether restorative repair was a good option for posterior teeth collected 10 years of follow-up data from a private dental clinic, they observed that the repair of posterior restorations reduced the annual failure rate from 4.1% to 2.9%. Similar results were found by Kanzow and Wiegand,[11] who concluded from their retrospective clinical study of 3239 patients that a repair can last as long as a complete replacement, so it can be considered a reliable alternative.

Despite the widespread use of minimally invasive dentistry, Mirsiaghi and others[12] showed that in their sample of clinicians, only 40% of 170 study participants used their knowledge of minimally invasive dentistry properly in their clinical routine. Previous studies[13,14] of dental practitioners found that many dentists (from the Dental Practice-Based Research Network [DPBRN])[15] often replace restorations that are not in optimal condition, regardless of their location, type of failure, number of surfaces, the material, used or time in the oral cavity. On the other hand, more recent studies have shown that some dental practitioners in the DPBRN have changed their clinical course over the years, with data suggesting an increase in repair options compared to replacement.[16,17]

Kanzow and others[18] performed a literature review based on 401 articles and noted that most schools of dentistry teach that repair should be performed to correct partial defects in restorations rather than replacing them completely. The same authors also observed that even though dental schools recommend the dental restoration repair procedure, approximately two-thirds of dental surgeons do not perform such procedures; however, they also reported that the proportion of dentists who perform repairs has increased over the years. Another study by Blum, Lynch, and Wilson[19] investigated data from 12 dental schools in Scandinavian countries (Norway, Denmark, Sweden, and Finland) using a questionnaire method. These authors showed that 11 out of 12 schools included the repair procedure in their primary curriculum. The only school that did not include repair in its curriculum intended to include it in the next five years. In a similar study, Brunton and others[20] assessed the teaching of the repair procedure in the academic environment at schools in Oceania (New Zealand, Australia, Fiji, and Papua New Guinea). All the universities investigated performed repairs and considered the treatment to have been successful, and 13 of 16 considered repair to be an appropriate alternative to complete replacement in their undergraduate courses.[20] Since all these studies demonstrate the importance of assessing the teaching of repair in the academic setting, quantifying its application in university clinics is necessary.

This study collected and analyzed data regarding the repair of dental restorations in patients treated in the clinics of a dental school over 10 years (2008 to 2017). The research hypothesis was that there would be no difference in the frequency of repairs of dental restorations according to the patient's age, tooth group and surface, and year. The clinical relevance of this study relates to the categorization and quantification of dental restoration repairs over time.

METHODS AND MATERIALS

This study was approved by the local research ethics committee (protocol 29695520.5.0000.5419). The data came from electronic dental records of patients

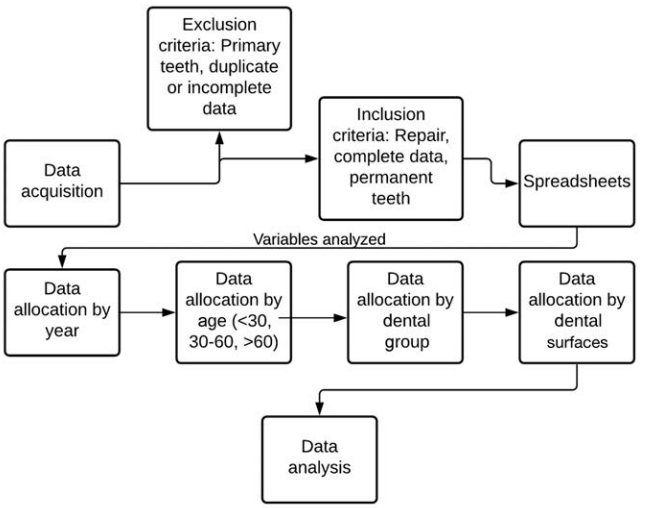

Figure 1. *Flowchart of the methods and processes performed in this study. The items above are related to each step in the Methods section of this article.*

that gave consent to use it. Figure 1 shows the study design.

Electronic dental records from 2008 to 2017 (10 years) were searched in the Romeu database (dental clinic software used at the School of Dentistry, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, which was built entirely based on free software solutions, such as PHP computer language and a PostgreSQL database management system (Romeu Database, FORP USP, Ribeirão Preto, São Paulo, Brazil). This database runs on any web browser and has been under development by the Informatic & Technology Team of the Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo (Ribeirão Preto, São Paulo, Brazil) since 2003. A specific search was conducted for procedures containing the word "restoration" in their description, "year," "age," "surface," "teeth," and "patient ID"; this search provided the initial sample. This sample was exported to a spreadsheet. Each line of the spreadsheet recorded the patient's identification number and age (at the time of treatment), the name of the procedure (eg, repair of restoration, resin restoration, amalgam restoration, ionomer restoration); the year in which the procedure was performed; and the tooth number and surface(s), along with the restoration report.

This study included electronic dental records with complete data, without duplication or filling errors, and that referred to the repair procedure for dental restorations performed on permanent teeth, always in composite resin. Electronic dental records with incomplete and duplicate data were excluded from this study, as well as dental records referring to procedures performed on primary teeth.

The following variables were analyzed: a) Repair by year: the number (absolute frequency) of repairs of dental restorations was recorded for each year, from 2008 to 2017; b) Repair by age group: allocated to the following groups-age up to 30 years old (<30), 30 to 60 years (30-60) and greater than 60 years (>60); c) Repair by tooth group: maxillary incisors, mandibular incisors, maxillary canines, mandibular canines, maxillary premolars, mandibular premolars, superior molars, and lower molars; d) Repair by tooth surface: allocated according to buccal, lingual, cervical, occlusal, incisal, mesial, and distal surfaces.

Data were analyzed with descriptive statistics, including the absolute and relative frequency of repairs by year (2008 to 2017), patients age group (<30, 30-60, >60), tooth group (incisors, canines, premolars, molars) and repaired dental surfaces (mesial, distal, buccal, lingual, occlusal, cervical). The chi-square test (95% confidence) compared the frequency of repairs between years, age group, and tooth groups and surfaces.

RESULTS

From the specific search for procedures containing the word "restoration" in their description, a total of 48,915 dental records and 53,436 procedures were found in the Romeu system from 2008 to 2017. Of these procedures, 34,115 surfaces were restored with composite resin, 8,845 with glass ionomer and 1,949 with amalgam. There were 7,566 teeth restored with temporary restorations and 34,277 with permanent restorations; 7,366 in primary teeth and 26,911 in permanent teeth. After excluding dental records that did not fit the inclusion criteria for the study, the repairs of dental restorations involved 1,408 surfaces and 828 teeth. The absolute and relative frequency of repairs of dental restorations per year, considering the age group, are shown in Figure 2, which also shows the percentage of repairs of dental restorations out of the total number of dental restorations performed, suggesting that replacement is gradually being replaced by repair.

Regarding the number of repairs according to dental groups, there were 446 incisor restoration repairs, 124 on canines, 310 premolars and 528 molars (Table 1). There were significant differences between canines versus incisors ($p<0.05$) and between canines vs. molars ($p<0.001$). No significant differences were found between incisors versus premolars, incisors versus molars, canines versus premolars and premolars versus molars. Table 1 also shows the frequency of repair by surfaces according to the dental groups. The frequency of repair

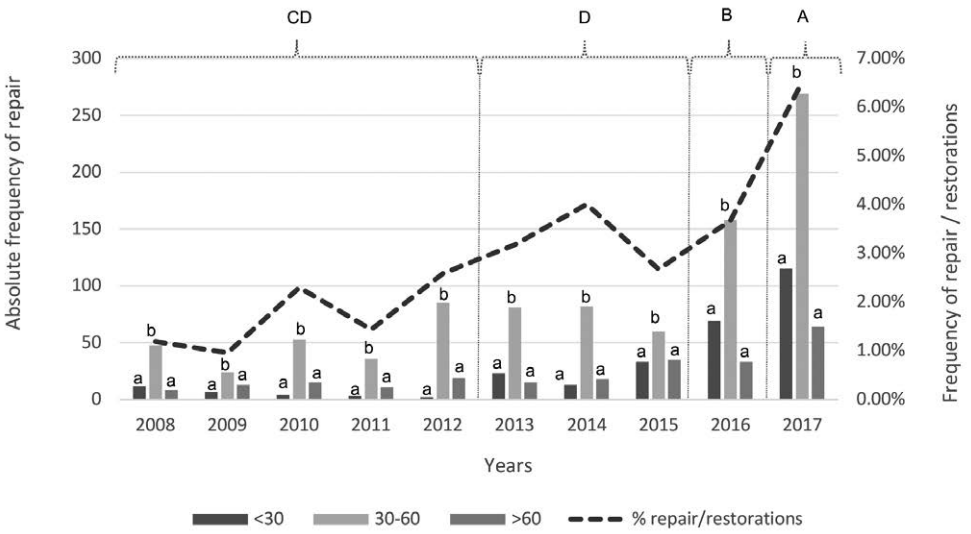

Figure 2. *The columns represent the absolute frequency of repairs of dental restorations per year according to the age group. The dashed line represents the frequency of repairs (% rate) in relation to the total number of restorative procedures. Different lowercase letters, at the columns, represent statistical differences between the age group (chi-square test,* p*<0.05). Different uppercase letters, at the middle part of the brackets, represent differences between the years (chi-square test,* p*<0.05).*

of incisal and lingual surfaces was similar ($p>0.05$), but both differed from the other surfaces ($p<0.001$).

DISCUSSION

This study showed that the frequency of repair of dental restorations increased significantly over the 10 years analyzed; furthermore, the frequency of repair differed significantly between age group, tooth group and surface. Consequently, there was no support for the research hypothesis that there were no differences among years or groups.

Considering the total number of repairs of dental restorations performed from 2008 to 2017, about 90% of the total teeth treated were repaired in 2015-2017. Interestingly, a significant increase in repairs was observed in 2015 and this continued in 2016 and 2017. It is worth mentioning that the rate of restorations/repairs of dental restorations also increased in these years, reflecting a shift in the balance between repair and replace, as shown in Figure 2. Considering the causes for such improvement in repair on dental restorations, we can cite the consistent scientific literature available in the period. For example, Fernandez and others[21] encourage performing repairs, both in resin and amalgam restorations, aiming to increase the longevity of the original restorations. Another study carried out by Martin and others[22] demonstrated that marginal sealing of restorations was a minimally invasive treatment that may be used instead of the replacement of restorations. Gordan and others[16] showed that when a defective restoration was repaired instead of replaced, a new intervention within the first year was less likely. Additionally, Javidi and others[2] observed a reduction in anxiety in patients who underwent repair treatment, which was also associated with a reduction in the

Table 1: *Absolute Frequency of Repair of Dental Restorations by Face and Tooth Group*[a]

	Incisors (a)	Canines (b)	Premolars (ab)	Molars (a)	∑ Faces
Buccal (A)	119	28	50	65	262
Lingual (B)	2	2	3	8	15
Palatal (A)	91	19	17	42	169
Mesial (A)	70	29	56	79	234
Distal (A)	45	18	58	66	187
Occlusal (A)	0	0	76	203	279
Incisal (B)	20	3	0	0	23
Cervical (A)	99	25	50	65	239
∑ Tooth	389	57	87	37	1408

[a]*Different lowercase letters represent significant differences between tooth groups (chi-square test,* p*<0.05). Different uppercase letters represent statistical similarity between the dental faces (chi-square test,* p*<0.001).*

amount of local anesthesia and less cavity preparation. Importantly, Moncada and others[23] concluded that a repair increases the useful life of the original restoration, even after a period of 10 years. Another reason that can justify the increased number of repairs is the decreased number of amalgam restorations that we observed in the electronic records. It is important to mention the Minamata Convention on Mercury's[24] scientific efforts not encouraging the use of dental materials such as amalgam, and with that, when amalgam restorations fail, for several reasons, dentists tend to use a medium or minimally invasive approach to rehabilitate the tooth.[25]

The data found in our study demonstrating the increase in the repair of dental restorations in the academic environment can be corroborated by Blum and others.[19] Although their study differed from ours as it was applied through questionnaires, they concluded that 91.67% of 12 Scandinavian dental schools have repair in their curriculum; Brunton and others [20] observed the same trend in 81% of the 16 schools in Oceania. Despite the acceptance and adoption of repair by dental schools, literature from the United States of America indicates that repairing dental restorations is not the first clinical choice in private dental clinics.[1] In Europe, a study of 1805 dentists by Kanzow and others [3] found that only 2.2% had never performed a repair, whereas the others had repaired restorations of different materials, with resin being the most frequent (93.4%), and others being ceramics, crowns, metallic restorations, and amalgams.

Concerning the repair of dental restorations according to age group, the highest incidence was observed in individuals aged 30 to 60 years. The same occurred for the total number of restorative procedures, with these individuals also receiving the highest number of restorations. Indeed, national epidemiological studies (Brazil) have shown that 35- to 44-year-old individuals have the highest number of teeth restored (7.33%), followed by 15-to-19-year-olds (2.16%) and 65-to-74-year-olds (1.62%);[26] this indicates that the teeth that are restored are lost as the individuals age, and the greater demand for adult dental services. This pattern (highest incidence in 30-60-year-olds) was also observed in other studies, such as that of Javidi and others,[2] in which adult patients represented the largest group in their sample, and both young and elderly individuals accounted for similar significantly smaller proportions of the sample, also consistent with the findings of this study. Van de Sande and others[9] also found that the average age of participants in their study was in the adult age group.

Regarding tooth group, the canines received the lowest frequency of repairs of their dental restorations during the study period, as also reported by Van de Sande and others[9] According to these authors, the incisal surface is considered at high risk of failure because it is subject to great masticatory stress, which indicates why the incisal surface was also one of the least repaired in our study. However, the lingual surface received a similar number of repairs. According to Wilson and others,[4] one of the criteria for performing a repair is esthetics. Thus, the lower number of lingual surface repairs may be related to a lower perception of esthetic failure by patients, which may have reduced the demand for the restorative service.

It is important to mention the role of electronic dental records (EDRs) in identifying trends in clinical decision-making; according to Schleyer and others,[27] 73.8% of DPBRN practitioners use a computer to record their clinical data. EDRs can be used to answer clinical questions, which could lead to improvements in patient care, thus creating a continuous cycle.[28] The process of storing data in EDRs can support analyses such as the one conducted in the current study. As EDR features and the training for filling the system are crucial to collect consistent data, it is worth mentioning how the EDR Romeu works. In the institution where the study was done, the professionals or students that see patients receive a login and password to access the system-that works only under the institutional internet protocol (IP)-and a broad training on how to feed the system (eg, patients' personal data, medical history, treatment plan, upload of images, procedures done). Considering the dental procedures, each has a code number. It is mandatory that together with the code, the tooth number and tooth surface be added. If by any chance the procedures are incorrectly registered, the responsible person sends a written message to the student (through the system) requesting him/her to correct them; in this case, the student is blocked to fill the system with other possible procedures until they correct the incorrect register. Consequently, for the procedure to be recorded in the system as a datum, the professor or person responsible for the clinic must log in and validate each procedure for each patient on a daily basis. In this sense, the major strength of this work was the analysis of data collected consistently over 10 years by a dental school, which indicated a probable scientific, evidence-based change in the way restorative procedures were being taught and practiced. Another contribution of this work is to have shown that the adoption of minimally invasive dentistry-constituted by repair of dental restorations-indeed increased in the institution. However, the rate of repairs per the total number of dental restorations was about 6% (Figure 2); in this context, a multicenter

analysis of similar data could assist the understanding of such a rate.

Nevertheless, EDRs have limitations compared to data collected in clinical trials.[27] For example, in this study the use of EDR data collected no information on the reason for repair (eg, correction of limited marginal openings and cavo marginal ditching, management of localized marginal staining, treatment of early lesions of secondary caries, repair of fractures that do not threaten the viability of the remaining restoration and tooth tissues, chipping of restoration margins, management of wear, correction of unacceptable esthetics, restoration of an endodontic access cavity prepared through an existing restoration).[4] Besides, this study was carried out in an academic setting, thus it is impossible to know whether the same behavior would occur outside the academic environment. Therefore, further studies should involve the assessment of restorative procedures in private dental clinics in the region in which this study was conducted.

CONCLUSIONS

The number of repairs of dental restorations increased over a 10-year period in the dental clinic of the School of Dentistry, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo from which the clinical data was collected, indicating an increase in the trend for minimally invasive dentistry in the academic field. Although it is a widely applicable procedure and has been used in all age groups, tooth groups and surfaces, when comparing frequencies between groups, those belonging to 30- to 60-years-of-age group received more repairs; the least repaired surfaces were the lingual and the incisal.

Acknowledgments

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Regulatory Statement

This study was conducted in accordance with all the provisions of the ethics committee of the Institutional Review Board of the University of São Paulo. The approval code issued for this study is CAAE: 29695520.5.0000.5419.

Conflict of Interest

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

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Literature Review

Spin in the Abstracts of Randomized Controlled Trials in Operative Dentistry: A Cross-sectional Analysis

X Fang • X Wu • C Levey • Z Chen • F Hua • L Zhang

Clinical Relevance

Readers and other stakeholders need to be aware of the existence of spin in RCT abstracts, and appraise the results and conclusions of RCT abstracts critically.

SUMMARY

Objective: To assess the presence and characteristics of spin in recently published RCT abstracts in operative dentistry and to investigate potential factors associated with the presence of spin.

Methods and Materials: The PubMed database was searched to identify parallel-group RCTs published between 2015 and 2019 in the field of operative dentistry, which compared two or more groups and had nonsignificant results for the primary outcome. Two authors evaluated independently the presence and characteristics of spin among these abstracts. Multivariable logistic regression analyses were conducted to identify factors associated with the presence of spin in the Results and the Conclusions sections, respectively.

Results: A total of 77 RCT abstracts were included,

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among which 58 (75.3%) showed at least one type of spin. Spin was identified in the Results and Conclusions sections of 32 (41.6%) and 45 (58.4%) abstracts, respectively. 19 RCTs (24.7%) presented spin in both the Results and the Conclusions section of abstracts. The presence of spin in the Results section of abstracts was significantly associated with source of funding (OR=8.10; *p*=0.025) and number of treatment arms was associated with the presence of spin in the Conclusions section of abstracts (OR=5.66; *p*=0.005).

Conclusion: The occurrence rate of spin in the sample of operative dentistry RCTs abstracts is high.

INTRODUCTION

In biomedical research, the randomized controlled trial (RCT) is the scientific gold standard for evaluating effectiveness of healthcare interventions.[1] The precise communication of RCT findings depends on accurate reporting of results.[2] Although relevant reporting guidelines such as CONSORT (CONsolidated Standards Of Reporting Trials) have been published,[3-5] various problems in published reports still exist.[6-8] One of the problems is that many authors have intentionally or unintentionally misrepresented and misinterpreted their findings, which could "spin" study results and mislead readers.[8]

In the medical literature, "spin" has been used to refer to "specific reporting strategies to distort the presentation and interpretation of results."[6] For instance, authors may divert readers' attention from nonsignificant primary outcomes to significant secondary endpoints. Spin was first systematically surveyed by Boutron and others[6] in biomedical literature with a representative sample of RCTs indexed in PubMed in December 2006 with nonstatistically significant primary outcomes. In their research, spin strategies were developed for the identification of spin in RCTs with nonsignificant primary outcomes, and spin was highly prevalent in the abstract's conclusions.[6] Thereafter, several studies found that spin is common in the biomedical literature, and the frequency of spin in abstracts varies (23% to 85%) by different studies.[7,9-15] In the field of dentistry, several studies have assessed the prevalence and type of spin in RCTs amongst high-impact dental research journals, endodontics, periodontology, and oral implantology and found that nearly 30.7%-85% of abstracts were identified as spin.[7,16,17]

Spin in abstracts may be a critical issue, as evidence has shown that abstracts are usually the first and the only part of an article that is accessible to clinicians,[18] and numerous clinicians make medical decisions based solely on the results and conclusions present in abstracts due to time constraints and unavailability of full-text articles.[19] However, to the best of our knowledge, spin has not been assessed in the field of operative dentistry.

Therefore, this study was conducted to (i) assess the existence and characteristics of spin in recently published RCT abstracts in operative dentistry; and (ii) investigate potential factors associated with the presence of spin in abstracts.

METHODS AND MATERIALS

Sample Creation

The PubMed database was searched using a combination of "Dentistry, Operative", "dental caries", "dental amalgam", "composite resins," and "dental cement" (for full search strategy; see Table 1), to identify RCT abstracts published during 2015-2019 in the field of operative dentistry, which compared two or more groups and have had nonsignificant result for the primary outcome(s).[6] Predefined inclusion criteria of RCTs were as follows: human participants, interventions associated with health care, experimental studies, presence of a control group, and random assignment of participants to the study or control group.[20,21] As determined *a priori*, RCTs for which the primary outcome could not be identified were excluded. To identify primary outcomes, the following potential sources were considered in order:[17]

1. Explicitly reported primary outcome(s) in the full text
2. The outcome used in sample size calculation
3. Explicitly reported primary outcome(s) in clinical trial registration
4. Outcome(s) reported in the *Results* section and consistent with the primary/main research objective

Data Extraction

The following information was extracted independently and in duplicate by two authors (XF and XW) from each of the included studies: continent of origin (first author), indexing in the Science Citation Index Expanded (SCIE) database (SCIE-indexed vs others), international collaboration, number of centers, number of treatment arms, trial registration, the topic of each study (materials and procedures), number of authors, statistician involvement, type of financial support, journal, and length of follow-up. Any disagreement was resolved through discussion.

Table 1: *The Search Strategy Used in This Study (Searched on June 29, 2020)*

Electronic Database	Search Strategy	Number of Hits
PubMed	#1 "Dentistry, Operative"[MeSH Terms] #2 dental caries[MeSH Terms] #3 dental amalgam[MeSH Terms] #4 composite resins[MeSH Terms] #5 dental cement[MeSH Terms] #6 #1 OR #2 OR #3 OR #4 OR #5 #7 randomized controlled trial[Publication Type] OR randomized controlled trials[MeSH Terms] OR "random allocation"[MeSH Terms] OR double-blind method[MeSH Terms] OR single-blind method[MeSH Terms]) OR ((single*[Text Word] OR doubl*[Text Word] OR trebl*[Text Word] OR tripl*[Text Word]) AND (mask*[Text Word] OR blind*[Text Word])) OR random*[Text Word] NOT ("review"[Publication Type] OR "meta-analysis"[Publication Type] OR "editorial"[Publication Type] OR "letter"[Publication Type] OR "comment"[Publication Type] OR "Case Reports"[Publication Type] #8 "2015/01/01"[PPDAT]: "2019/12/31"[PPDAT] #9 #6 AND #7 AND #8	1876

Evaluation of Spin

All eligible abstracts were collated into a Word document. Meanwhile, the journal titles, author names, and author affiliations were removed to guarantee blinded assessment of the presence and strategy of spin.[7] Calibration was conducted in iterative rounds of 10 randomly selected abstracts, until the agreement between the assessors (XF and FH) was excellent or better (Cohen κ>0.75). Thereafter, two assessors (XF and FH) evaluated the existence of spin and spin strategies for each included abstract independently and in duplicate. Any disagreement was resolved through discussion with the other authors.

Spin was evaluated in the *Results* section and the *Conclusions* section of the abstracts, respectively. A classification system was adapted from the one used by Boutron and others[6]; types of spin was classified into one of the following strategies:

- Focusing on statistically significant results (secondary endpoints, subgroup analysis, within-group comparison)
- Interpreting statistically nonsignificant results as equivalent
- Claiming benefit for statistically nonsignificant results
- Recommendation to use the treatment
- Focusing on a statistically significant primary outcome when there are several co-primary outcomes
- Verbiage implying numerical significance or "trend towards significance"

Statistical Analyses

Descriptive statistics were used to describe the existence and strategy of spin, as well as the percentage of spin by characteristics. Multivariable logistic regression analyses were conducted to detect the association between the presence of spin in the *Results* and *Conclusion* sections (dependent variables) and the extracted factors, namely continent of origin (first author), SCIE indexing, international collaboration, number of centers, number of treatment arms, the topic of each study (materials and procedures), trial registration, number of authors, statistician involvement, type of financial support, and length of follow-up. The goodness of fit was tested by Hosmer-Lemeshow test. For all statistical analyses, a two-sided p<0.05 was set as the criterion for statistical significance.

RESULTS

Characteristics of Included Abstracts

Figure 1 demonstrates the literature flow of this study. A total of 77 RCT abstracts with statistically nonsignificant results for the primary outcomes were included in this study (Table 2). Table 3 describes the characteristics of included abstracts. Amongst the 77 RCTs, 33 were from South America, followed by Europe (n=18), Asia

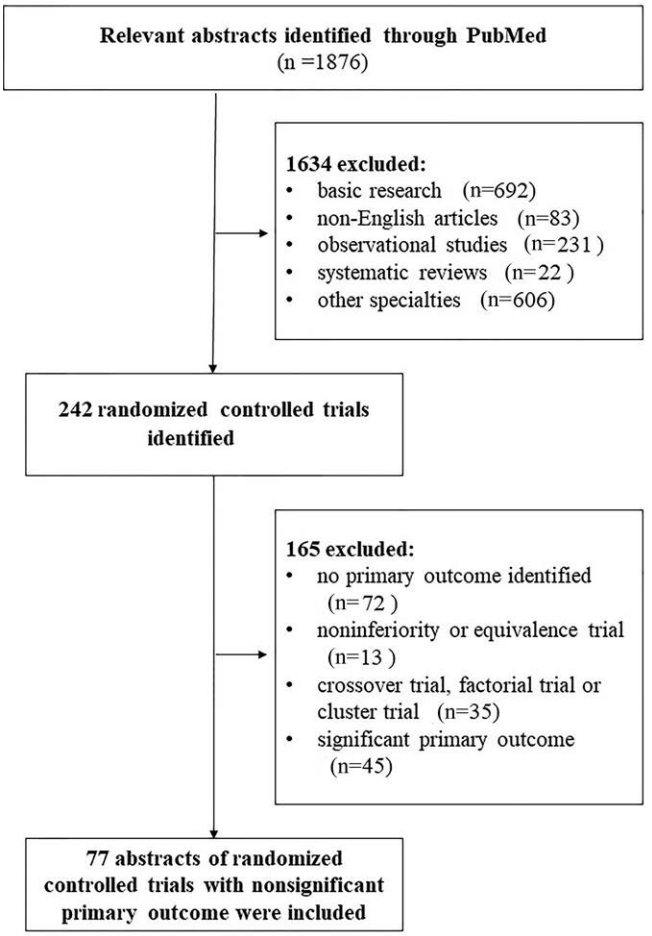

Figure 1. *Flowchart of the included abstracts.*

(n=17), and others (n=9). Most RCTs were indexed in SCIE (79.2%), conducted in a single center (89.6%), focused on dental materials (adhesives, composite resin, amalgam, and others) (63.6%) and without international collaboration (62.3%). Approximately, a third of the RCTs focused on procedures, such as placement techniques, adhesive application modes, different subgingival restorative margin designs, and others. More than half of the RCTs were two-armed trials (50.6%), with the number of authors being four to seven (72.7%). The source of funding was industry in 14 (18.2%) RCTs, other sources in 38 (49.4%), and unreported/unfunded in 25 (32.5%). Only 18 studies reported the involvement of statisticians. The number of RCTs were mainly published in *Journal of Dentistry* (23.4%), *Clinical Oral Investigations* (22.1%), and *Operative Dentistry* (13.0%). More than half of the trials (68.8%) were at 1-3 year length of follow-up, 16 were at less than 1-year follow-up, and only 8 were followed for more than 3 years.

Spin Assessment

Spin was identified in 58 (75.3%) of the 77 included abstracts. Nineteen RCTs (24.7%) presented spin in both the *Results* and *Conclusions* section of abstracts, and more than one type of spin strategy was found in 25 (32.5%) abstracts. Frequencies of each spin strategy are presented in Table 4.

Spin was observed in the *Results* section of 32 (41.6%) abstracts. The most frequent spin strategies in the *Results* section were focusing on significant within-group comparisons for primary outcomes (17, 22.1%) and focusing on a statistically significant primary outcome when there are several co-primary outcomes (10, 13.0%).

The *Conclusions* section of 45 (58.4%) abstracts presented with spin. Claiming equivalence/noninferior/comparable/similar for statistically nonsignificant results was the most common spin strategy in the *Conclusions* section (16, 20.8%), followed by focusing on a statistically significant primary outcome when there are several co-primary outcomes (13, 16.9%) and focusing on only statistically significant results (ie, secondary endpoints, subgroup analysis, and within-group analysis) (11, 14.3%). Other spin strategies identified included claiming benefit with no recognition of the statistically nonsignificant results for the primary outcome (5, 6.5%), acknowledge statistically nonsignificant results for the primary outcome but emphasize the beneficial effect of treatment (4, 5.2%), and recommendation to use the treatment (4, 5.2%).

Predictors of Spin

In the multivariable logistic analyses, RCTs with unreported/unfunded source of funding were significantly more likely to present spin in the *Results* section of abstracts (OR=8.10, 95% CI: 1.31-50.16; p=0.025), and RCTs with more than two treatment arms were significantly more likely to present spin in the *Conclusions* section of abstracts (OR=5.66, 95% CI: 1.71-18.80; p=0.005). Other factors, namely SCIE indexing, international collaboration, number of centers, the topic of each study (materials and procedures), trial registration, number of authors, statistician involvement, continent of origin, and length of follow-up were not significantly associated with the presence of spin (p>0.05) (Table 5).

DISCUSSION

This is the first study to evaluate spin and analyze factors associated with the presence of spin in the field of operative dentistry. We concentrated our analysis on the abstracts of RCTs (with statistically nonsignificant

Table 2: *A List of Included RCTs in the Study*

Serial Number	Title of Article	Journal	Digital Object Identifier (DOI)
1	Bonding performance of simplified adhesive systems in noncarious cervical lesions at 2-year follow-up: A double-blind randomized clinical trial	*Operative Dentistry*	10.2341/18-049-c
2	Clinical, double blind, randomized controlled trial of experimental adhesive protocols in caries-affected dentin	*Clinical Oral Investigations*	10.1007/s00784-018-2615-7
3	An 18-month clinical evaluation of three different universal adhesives used with a universal flowable composite resin in the restoration of noncarious cervical lesions	*Clinical Oral Investigations*	10.1007/s00784-018-2571-2
4	Twenty-four-month clinical performance of different universal adhesives in etch-and-rinse, selective etching and self-etch application modes in NCCL - a randomized controlled clinical trial	*Journal of Applied Oral Science*	10.1590/1678-7757-2018-0358
5	Microcavitated (ICDAS 3) carious lesion arrest with resin or glass ionomer sealants in first permanent molars: A randomized controlled trial	*Journal of Dentistry*	10.1016/j.jdent.2019.07.001
6	Randomized clinical trial of class II restoration in permanent teeth comparing ART with composite resin after 12 months	*Clinical Oral Investigations*	10.1007/s00784-018-2787-1
7	18-month clinical evaluation of a copper-containing universal adhesive in noncarious cervical lesions: A double-blind, randomized controlled trial	*Journal of Dentistry*	10.1016/j.jdent.2019.103219
8	Randomized 36-month follow-up of posterior bulk-filled resin composite restorations	*Journal of Dentistry*	10.1016/j.jdent.2019.05.018
9	Fluoride-releasing effect of a modified resin denture containing S-PRG fillers on salivary fluoride retention: A randomized clinical study	*Caries Research*	10.1159/000490627
10	Retention of moisture-tolerant fluoride-releasing sealant and amorphous calcium phosphate-containing sealant in 6-9-year-old children: A randomized controlled trial	*Journal of Indian Society of Pedodontics and Preventive Dentistry*	10.4103/jisppd.jisppd_173_18
11	Evaluation of the efficacy of calcium silicate vs. glass ionomer cement indirect pulp capping and restoration assessment criteria: a randomised controlled clinical trial-2-year results	*Clinical Oral Investigations*	10.1007/s00784-018-2638-0
12	Esthetic improvements of postorthodontic white-spot lesions treated with resin infiltration and microabrasion: A split-mouth, randomized clinical trial	*Angle Orthodontist*	10.2319/041218-274.1

Table 2: *A List of Included RCTs in the Study (cont.)*

Serial Number	Title of Article	Journal	Digital Object Identifier (DOI)
13	Clinical evaluation of a low-shrinkage resin composite in endodontically treated premolars: 3-year follow-up	*Clinical Oral Investigations*	10.1007/s00784-018-2677-6
14	Atraumatic restorative treatment-ART in early childhood caries in babies: 4 years of randomized clinical trial	*Clinical Oral Investigations*	10.1007/s00784-019-02800-8
15	Effects of orthodontic treatment and different fluoride regimens on numbers of cariogenic bacteria and caries risk: A randomized controlled trial	*European Journal of Orthodontics*	10.1093/ejo/cjy025
16	A randomized controlled clinical trial of glass carbomer restorations in Class II cavities in primary molars: 12-month results	*Quintessence International*	10.3290/j.qi.a42573
17	A clinical and radiographic investigation comparing the efficacy of cast metal and indirect resin onlays in rehabilitation of permanent first molars affected with severe molar incisor hypomineralisation (MIH): A 36-month randomised controlled clinical trial	*European Archives of Paediatric Dentistry*	10.1007/s40368-019-00430-y
18	Comparative evaluation of resin infiltration and remineralisation of noncavitated smooth surface caries lesions: 6-month results	*Oral health & Preventive Dentistry*	10.3290/j.ohpd.a42203
19	An RCT of atraumatic restorative treatment for older adults: 5 year results	*Journal of Dentistry*	10.1016/j.jdent.2019.03.003
20	One-year clinical evaluation of bulk-fill flowable vs. regular nanofilled composite in noncarious cervical lesions	*Clinical Oral Investigations*	10.1007/s00784-018-2509-8
21	Retention and remineralization effect of moisture tolerant resin-based sealant and glass ionomer sealant on noncavitated pit and fissure caries: Randomized controlled clinical trial	*Journal of Dentistry*	10.1016/j.jdent.2019.05.027
22	Clinical follow-up of a fissure sealant placed using different adhesive protocols: A 24-month split-mouth study	*Operative Dentistry*	10.2341/17-055-c
23	Alternative caries management options for primary molars: 2.5-year outcomes of a randomised clinical trial	*Caries Research*	10.1159/000477855
24	Eighteen-month clinical study of universal adhesives in noncarious cervical lesions	*Operative Dentistry*	10.2341/16-320-c
25	MI Varnish and MI Paste Plus in a caries prevention and remineralization study: A randomized controlled trial	*Clinical Oral Investigations*	10.1007/s00784-017-2314-9
26	Influence of surface treatment on the performance of silorane-based composite resin in class I restorations: A randomized clinical trial	*Clinical Oral Investigations*	10.1007/s00784-018-2390-5

Table 2: *A List of Included RCTs in the Study (cont.)*

Serial Number	Title of Article	Journal	Digital Object Identifier (DOI)
27	Effect of dentin roughness on the adhesive performance in noncarious cervical lesions: A double-blind randomized clinical trial	*Journal of Dentistry*	10.1016/j.jdent.2017.09.011
28	Use of casein amorphous calcium phosphate (CPP-ACP) on white-spot lesions: Randomised clinical trial	*Oral health & preventive dentistry*	10.3290/j.ohpd.a39749
29	Selective vs stepwise removal of deep carious lesions in primary molars: 12-months results of a randomized controlled pilot trial	*Journal of Dentistry*	10.1016/j.jdent.2018.07.011
30	Caries arrest by topical fluorides in preschool children: 30-month results	*Journal of Dentistry*	10.1016/j.jdent.2017.12.013
31	Comparison of resin modified glass ionomer cement and composite resin in class II primary molar restorations: A 2-year parallel randomised clinical trial	*European Archives of Paediatric Dentistry*	10.1007/s40368-018-0371-7
32	Efficacy of sealing occlusal caries with a flowable composite in primary molars: A 2-year randomized controlled clinical trial	*Journal of Dentistry*	1016/j.jdent.2018.05.014
33	Microbial load after selective and complete caries removal in permanent molars: A randomized clinical trial	*Brazilian Dental Journal*	10.1590/0103-6440201801816
34	Proximal carious lesions infiltration-a 3-year follow-up study of a randomized controlled clinical trial	*Clinical Oral Investigations*	10.1007/s00784-017-2135-x
35	A randomized controlled trial of caries prevention in dental practice	*Journal of Dental Research*	10.1177/0022034517702330
36	Low-cost GICs reduce survival rate in occlusal ART restorations in primary molars after one year: A RCT	*Journal of Dentistry*	10.1016/j.jdent.2016.12.006
37	Impact of the intermediary layer on sealant retention: A randomized 24-month clinical trial	*Clinical Oral Investigations*	10.1007/s00784-016-1890-4
38	Effectiveness of pretreatment with chlorhexidine in restoration retention: A 36-month follow-up randomized clinical trial	*Journal of Dentistry*	10.1016/j.jdent.2017.02.014
39	Eighteen-month clinical performance of composite resin restorations with two different adhesive systems for molars affected by molar incisor hypomineralization	*Clinical Oral Investigations*	10.1007/s00784-016-1968-z
40	Effectiveness of pit and fissure sealants bonded with different adhesive systems: A prospective randomized controlled trial	*Clinical Oral Investigations*	10.1007/s00784-016-2016-8
41	Influence of adhesive type and placement technique on postoperative sensitivity in posterior composite restorations	*Operative Dentistry*	10.2341/16-010-c

Table 2: *A List of Included RCTs in the Study (cont.)*

Serial Number	Title of Article	Journal	Digital Object Identifier (DOI)
42	No additional benefit of using a calcium hydroxide liner during stepwise caries removal: A randomized clinical trial	*Journal of the American Dental Association*	10.1016/j.adaj.2017.02.019
43	Sealing composite with defective margins, good care or over treatment? Results of a 10-year clinical trial	*Operative Dentistry*	10.2341/14-143-c
44	Can repair increase the longevity of composite resins? Results of a 10-year clinical trial	*Journal of Dentistry*	10.1016/j.jdent.2014.05.015
45	Effect of a chlorhexidine/thymol and a fluoride varnish on caries development in erupting permanent molars: A comparative study	*European Archives of Paediatric Dentistry*	10.1007/s40368-015-0192-x
46	Dentin hypersensitivity treatment of noncarious cervical lesions - a single-blind, split-mouth study	*Brazilian Oral Research*	10.1590/1807-3107BOR-2015.vol29.0045
47	Four-year randomized clinical trial to evaluate the clinical performance of a glass ionomer restorative system	*Operative Dentistry*	10.2341/13-239-c
48	A three-year clinical evaluation of a one-step self-etch and a two-step etch-and-rinse adhesive in noncarious cervical lesions	*Journal of Dentistry*	10.1016/j.jdent.2014.12.009
49	Clinical and radiographic assessment of the efficacy of calcium silicate indirect pulp capping: A randomized controlled clinical trial	*Journal of Dental Research*	10.1177/0022034515571415
50	A prospective randomized clinical trial into the capacity of a toothpaste containing NovaMin to prevent white spot lesions and gingivitis during orthodontic treatment	*Progress in Orthodontics*	10.1186/s40510-015-0095-8
51	Two-year randomized, controlled clinical trial of a flowable and conventional composite in Class I restorations	*Operative Dentistry*	10.2341/15-038-c
52	A new universal simplified adhesive: 36-month randomized double-blind clinical trial	*Journal of Dentistry*	10.1016/j.jdent.2015.07.005
53	Influence of isolation method of the operative field on gingival damage, patients' preference, and restoration retention in noncarious cervical lesions	*Operative Dentistry*	10.2341/14-089-c
54	Efficacy of fluoride varnish and casein phosphopeptide-amorphous calcium phosphate for remineralization of primary teeth: a randomized clinical trial	*Medical Principles and Practice*	10.1159/000379750
55	Effect of pretreatment with chlorhexidine on the retention of restorations: A randomized controlled trial	*Brazilian Dental Journal*	10.1590/0103-6440201300009

Table 2: *A List of Included RCTs in the Study (cont.)*

Serial Number	Title of Article	Journal	Digital Object Identifier (DOI)
56	Five-year evaluation of a low-shrinkage Silorane resin composite material: A randomized clinical trial	*Clinical Oral Investigations*	10.1007/s00784-014-1238-x
57	Randomized *in vivo* evaluation of photodynamic antimicrobial chemotherapy on deciduous carious dentin	*Journal of Biomedical Optics*	10.1117/1.jbo.20.10.108003
58	Six-year clinical performance of etch-and-rinse and self-etch adhesives	*Dental Materials*	10.1016/j.dental.2016.06.003
59	Milk sweetened with Xylitol: A proof-of-principle caries prevention randomized clinical trial	*Journal of Dentistry for Children*	—
60	Long-term effect of Erythritol on dental caries development during childhood: A posttreatment survival analysis	*Caries Research*	10.1159/000450762
61	Anticaries effect of low-fluoride dentifrices with phosphates in children: A randomized, controlled trial	*Journal of Dentistry*	10.1016/j.jdent.2016.04.013
62	Efficacy of a new sealant to prevent white spot lesions during fixed orthodontic treatment: A 12-month, single-center, randomized controlled clinical trial	*Journal of Orofacial Orthopedics*	10.1007/s00056-016-0052-2
63	Nutrient supplementation may adversely affect maternal oral health--a randomised controlled trial in rural Malawi	*Maternal and Child Nutrition*	10.1111/mcn.12204
64	Bilayer technique and nano-filled coating increase success of approximal ART restorations: A randomized clinical trial	*International Journal of Paediatric Dentistry*	10.1111/ipd.12194
65	Randomized clinical trial on arresting dental root caries through silver diammine fluoride applications in community-dwelling elders	*Journal of Dentistry*	10.1016/j.jdent.2016.05.005
66	Comparison of oral health education and fluoride varnish to prevent early childhood caries: A randomized clinical trial	*Caries Research*	10.1159/000446877
67	Quantitative Light-induced Fluorescence-digital as an oral hygiene evaluation tool to assess plaque accumulation and enamel demineralization in orthodontics	*Angle Orthodontist*	10.2319/092415-648.1
68	Periodontal response to two different subgingival restorative margin designs: A 12-month randomized clinical trial	*Clinical Oral Investigations*	10.1007/s00784-015-1616-z
69	Two-year randomized clinical trial of self-etching adhesives and selective enamel etching	*Operative Dentistry*	10.2341/15-130-c
70	Effects of various remineralizing agents on the outcome of postorthodontic white spot lesions (WSLs): A clinical trial	*Progress in Orthodontics*	10.1186/s40510-016-0138-9

Table 2: *A List of Included RCTs in the Study (cont.)*

Serial Number	Title of Article	Journal	Digital Object Identifier (DOI)
71	Anti-microbial efficacy of green tea and chlorhexidine mouth rinses against streptococcus mutans, lactobacilli spp. and candida albicans in children with severe early childhood caries: A randomized clinical study	*Journal of Indian Society of Pedodontics and Preventive Dentistry*	10.4103/0970-4388.175518
72	A randomised controlled trial to measure the effects and costs of a dental caries prevention regime for young children attending primary care dental services: The Northern Ireland Caries Prevention In Practice (NIC-PIP) trial	*Health Technology Assessment*	10.3310/hta20710
73	Controlled, prospective, randomized, clinical split-mouth evaluation of partial ceramic crowns luted with a new, universal adhesive system/resin cement: Results after 18 months	*Clinical Oral Investigations*	10.1007/s00784-016-1779-2
74	Efficacy of 30% silver diamine fluoride compared to atraumatic restorative treatment on dentine caries arrestment in primary molars of preschool children: A 12-months parallel randomized controlled clinical trial	*Journal of Dentistry*	10.1016/j.jdent.2019.07.003
75	Randomized clinical trial on the survival of lithium disilicate posterior partial restorations bonded using immediate or delayed dentin sealing after 3 years of function	*Journal of Dentistry*	10.1016/j.jdent.2019.02.001
76	Effect of refurbishing amalgam and resin composite restorations after 12 years: Controlled clinical trial	*Operative Dentistry*	10.2341/16-267-cr
77	Clinical evaluation of the efficacy of one self-adhesive composite in dental hypersensitivity	*Clinical Oral Investigations*	10.1007/s00784-014-1390-3

primary outcomes) published from 2015 to 2019. Spin was identified in 75.3% abstracts in one form or another, which exceeded the occurrence of spin in most of the studies (30.7%-70%)[11,12,16] but was less than that identified in endodontic RCTs (85.0%).[7] Some explanations for the difference among studies may be RCTs from different specialties, diverse instruments for evaluation of spin (the Boutron instrument or its variations), and some bias in assessment among investigators. The spin strategies manifest in various ways. The most common strategy in the *Results* section was focusing on significant within-group comparisons for primary outcomes rather than between-group comparisons. While claiming equivalence for statistically nonsignificant results was the most frequent spin strategy in the *Conclusions* section. Spin was more prevalent in the *Conclusions* section (58.4%) than *the Results* section (41.6%). Previous studies also reported that the *Conclusion* sections of abstracts were more susceptible to spin than the other sections.[7,10]

Previous studies have assessed whether particular factors were associated with the presence of spin, including financial support, journal impact factor, intervention type, trial phase, trial type, statistician involvement, number of authors, sample size, international collaboration, number of centers, the number of treatment arms, reporting of trial registration, article citations, and the conflict-of-interest disclosures.[10,12,14-17] Amongst these, most of the studies consistently found no factors to be significantly associated with spin,[10,12,14-16] but only one study[17] concluded that multicenter RCTs were less likely to present spin in abstracts. In this study, the presence of spin in the *Results* section of abstracts was significantly associated with source of funding, which was inconsistent with the previous studies.[10,16] Nonindustry-

Table 3: *Characteristics of Included RCT Abstracts (N=77)*

Characteristics	Number of Abstracts n (%)
Topic	
Materials	49 (63.6)
Procedures	28 (36.4)
International collaboration	
Yes	29 (37.7)
No	48 (62.3)
Continent of origin	
Europe	18 (23.4)
Asia	17 (22.1)
South America	33 (42.9)
Others	9 (11.7)
SCIE indexing	
Indexed in SCIE	61 (79.2)
Not indexed in SCIE	16 (20.8)
Funding source	
Industry	14 (18.2)
Other sources	38 (49.4)
Unfunded/Unreported	25 (32.5)
Centers	
Single center	69 (89.6)
Multicenters	8 (10.4)
Number of treatment arms	
Two arms	39 (50.6)
≥Three arms	38 (49.4)
Number of authors	
<4	7 (9.1)
4-7	56 (72.7)
>7	14 (18.2)
Statistician	
Yes	18 (23.4)
No	59 (76.6)
Trial registration	
Yes	50 (64.9)
No	27 (35.1)
Length of follow-up	
<1 year	16 (20.8)
1-3 years	53 (68.8)
>3 years	8 (10.4)
Journal	
Journal of Dentistry	18 (23.4)
Clinical Oral Investigations	17 (22.1)
Operative Dentistry	10 (13.0)
Caries Research	4 (5.2)
Others	28 (36.4)
Total	77(100.0)

sponsored RCTs may lack specialized teams with the involvement of methodological experts and statistician, or they may be exploratory research with small sample sizes due to limited financial support. Therefore, more statistically nonsignificant results may be found, and spin was more likely to occur. It should also be noted that the difference among studies may be explained by different subjects or sampling, and further study should be conducted to draw conclusions. Furthermore, number of treatment arms was associated with the presence of spin in the *Conclusions* section of abstracts. RCTs with more than two arms were more likely to focus only on significant between-group results, which was consistent with the findings that focusing on a statistically significant primary outcome when there are several co-primary outcomes (13, 16.9%) was a common spin strategy in the *Conclusions* section.

Spin in abstracts is particularly crucial, because readers often focus on this concise summary to determine whether the literature is worthy of full-text review. Distorted results can affect researchers'/clinicians' interpretation of the experimental treatment. Boutron and others[22] conducted an RCT to evaluate the impact of spin in abstracts of cancer RCTs on clinicians' interpretation of treatment benefit. When abstracts present with spin, readers considered the treatment as more beneficial to patients (mean difference, 0.71; 95% CI, 0.07-1.35; p=0.030), and clinicians were more interested in reading the full text (mean difference, 0.77; 95% CI, 0.08-1.47; p=0.029). Furthermore, RCTs with spin in abstracts were more likely to be cited compared with those without spin.[17] Spin may not only mislead readers by distorting results but also exert adverse influence on further research.[23] This study might raise awareness among the readers of operative dentistry about spin in published reports.

Manuscripts with statistically significant results are more likely to be published.[24] This common phenomenon may prompt some researchers intentionally or subconsciously to spin results and conclusions in order to attract peer reviewer attention.[8] Reporting guidelines like CONSORT 2010 were developed to help authors improve the reporting quality of manuscripts.[4,20,21] However, guidelines on avoiding spin are not available. Thus, present guidelines need to be expanded to minimize the occurrence of spin. Peer reviewers and editors should be aware of spin in abstract reporting and provided with specific instruments to help identify it in manuscripts. Manuscript authors are supposed to report and interpret results objectively, and so guidelines for authors should also reflect this.

This study has several strengths and limitations. First, 5 years of published RCTs in the field of operative

Table 4: *Frequencies of Spin Strategies in the Results and Conclusions Section (N=77)*

Spin in the Results Section	**N (%)**
Focusing on significant within-group comparisons for primary outcomes	17 (22.1%)
Focusing on significant within- and/or between-group secondary outcomes	9 (11.7%)
Focusing on a statistically significant primary outcome when there are several co-primary outcomes	10 (13.0%)
Verbiage implying numerical significance or "trend towards significance"	2 (2.6%)
Spin in the conclusions section	**N (%)**
Claiming equivalence/noninferior/comparable/similar for statistically nonsignificant results	16 (20.8%)
Claiming benefit with no recognition of the statistically nonsignificant results for the primary outcome	5 (6.5%)
Focusing on only statistically significant results (i.e., secondary endpoints, subgroup analysis, within-group analysis)	11 (14.3%)
Acknowledge statistically nonsignificant results for the primary outcome but emphasize the beneficial effect of treatment	4 (5.2%)
Focusing on a statistically significant primary outcome when there are several co-primary outcomes	13 (16.9%)
Recommendation to use the treatment	4 (5.2%)

Table 5: *Binary Logistic Regression-derived OR and 95% CI, with Presence of Spin in the Results and the Conclusions Sections as the Dependent Variables for the Included 77 Abstracts*

Predictor	**Presence of Spin**			
	In the Results Section[a]		**In the Conclusions Section[b]**	
	OR (95% CI)	***p*-value[c]**	**OR (95% CI)**	***p*-value[c]**
SCIE-indexed journal				
No	Reference		Reference	
Yes	1.99 (0.38, 10.36)	0.414	0.31 (0.05, 1.81)	0.192
Centers				
Single center	Reference		Reference	
Multicenter	1.29 (0.20, 8.21)	0.791	1.11 (0.18, 6.90)	0.914
Number of treatment arms				
Two arms	Reference		Reference	
≥Three arms	1.15 (0.36, 3.71)	0.816	5.66 (1.71, 18.80)	**0.005**
International collaboration				
No	Reference		Reference	
Yes	2.14 (0.57, 8.04)	0.258	0.64 (0.19, 2.22)	0.484
Number of authors	1 author		1 author	
	0.92 (0.67, 1.28)	0.631	1.14 (0.84, 1.54)	0.411
Continent of origin				
Europe	Reference		Reference	
Asia	1.24 (0.17, 9.19)	0.832	2.87 (0.40, 20.76)	0.296
South America	1.79 (0.33, 9.76)	0.499	1.60 (0.38, 6.75)	0.522
Others	2.07 (0.31, 13.81)	0.454	2.01 (0.29, 14.21)	0.483
Statistician				
No	Reference		Reference	
Yes	2.82 (0.68, 11.80)	0.155	1.11 (0.28, 4.37)	0.880

Table 5: *Binary Logistic Regression-derived OR and 95% CI, with Presence of Spin in the Results and the Conclusions Sections as the Dependent Variables for the Included 77 Abstracts (cont.)*

Predictor	Presence of Spin			
	In the Results Section[a]		In the Conclusions Section[b]	
	OR (95% CI)	*p*-value[c]	OR (95% CI)	*p*-value[c]
Topic				
Procedures	Reference		Reference	
Materials	4.76 (0.89, 25.50)	0.068	0.45 (0.11, 1.83)	0.264
Trial registration				
No	Reference		Reference	
Yes	0.99 (0.29, 3.40)	0.984	1.17 (0.34, 4.06)	0.801
Funding source				
Industry	Reference		Reference	
Other sources	1.20 (0.26, 5.63)	0.814	1.47 (0.36, 6.01)	0.594
Unfunded/Unreported	8.10 (1.31, 50.16)	**0.025**	0.78 (0.13, 4.55)	0.781
Length of follow-up				
<1 year	Reference		Reference	
1-3 years	0.26 (0.04, 1.52)	0.135	3.36 (0.57, 19.85)	0.182
>3 years	0.20 (0.20, 1.96)	0.166	7.40 (0.62, 88.67)	0.114

[a] *Model summary: dependent variable coding: [0] no spin in the Results section, [1] with spin in the Results section;* p *(Hosmer and Lemeshow) = 0.676;* R^2 *(Nagelkerke) = 0.353.*

[b] *Model summary: Dependent variable coding: [0] no spin in the Conclusions section, [1] with spin in the Conclusions section;* p *(Hosmer and Lemeshow) = 0.733;* R^2 *(Nagelkerke) = 0.258.*

[c] *Statistically significant* p*-values (<0.05) are provided in bold.*

dentistry were evaluated to provide a comprehensive view of the issues related to the spin in this field. Second, as far as we know, this is the first study to investigate potential factors associated with presence of spin in the *Results* and *Conclusions* sections, respectively. One limitation of this study is that spin was not evaluated in the main text of included RCTs. A recent study assessed spin in the abstract and the full text of dental RCTs, and found 78.7% of the included RCTs presented some type of spin in the main text.[14] However, the small sample size may not be able to provide highly accurate results. Further study is needed to identify spin in the full texts of dental literature.

CONCLUSIONS

The occurrence rate of spin (75.3%) in the sample of operative dentistry RCTs abstracts is high. Source of funding (OR=8.10; p=0.025) and number of treatment arms (OR=5.66; p=0.005) were associated with the presence of spin in the *Results* and the *Conclusions* sections of abstracts, respectively. Readers and other stakeholders need to be aware of the existence of spin in RCT abstracts, and appraise the results and conclusions of RCT abstracts critically.

Acknowledgments

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Conflict of Interest

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

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Laboratory Research

Tooth Color Change and Erosion: Hydrogen Peroxide Versus Non-peroxide Whitening Strips

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Clinical Relevance

There are uncertainties about non-peroxide strip whitening efficacy and effect on enamel erosion. This study indicates that peroxide whitening strips have superior whitening efficacy compared to non-peroxide strips. None of the tested products caused concerning enamel erosion.

SUMMARY

Aim: The study evaluated the efficacy and potential erosion of non-peroxide strips compared to hydrogen peroxide (HP) whitening strips (WSs).

Methods: Color evaluation samples (N=64) were distributed into four groups and treated according to manufacturer's directions. NC: Negative control treated with water; BT: Non-peroxide Brilliant Dissolving Strips; FM: Non-peroxide Fancymay Teeth WSs; WS: Crest 3D Brilliance HP White Strips. A contact-type spectrophotometer was used to measure color at baseline (T1), 1-day posttreatment (T2), and 1-week posttreatment (T3). Teeth were cut to a rectangular block for micro-CT erosion assessment. The samples (N=30) were divided into five groups. In addition to the four groups for color assessment, a positive control (PC) treated with 0.25% citric acid was added. The samples were scanned, reconstructed, and measured for erosion depth using a micro-CT analysis program software. Kruskal-Wallis test was used to determine differences in color change and erosion depth among the groups. Tests of

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hypotheses were two-sided with an alpha level of 0.05.

Results: The mean ΔE^*_{ab} at 1-day/1-week posttreatment were 2.4/2.5, 2.8/2.9, 2.8/3.2, and 8.6/11.0 for NC, BT, FM, and WS, respectively. There was a statistically significant difference for ΔE^*_{ab} at 1-day and 1-week posttreatment ($p<0.001$). Group WS had the highest color change, while the other three groups did not differ from each other ($p>0.05$). Mean erosion depths in microns were 0.52, 0.58, 0.42, 0.49, and 29.55 for NC, BT, FM, WS, and PC, respectively. There was a statistically significant difference among the groups ($p=0.004$). Group PC had the greatest erosion, while the other groups had negligible erosion that did not differ from each other ($p>0.05$).

Conclusion: Peroxide WSs had superior whitening efficacy compared to non-peroxide strips. None of the tested products compromised tooth structure integrity through enamel erosion.

INTRODUCTION

The desire to have a sparkling and white smile has driven the growth of the global whitening market. In 2019, the tooth whitening market was valued at $1.7 billion in the United States, and is further expected to grow substantially to exceed $2 billion in 2024.[1] Historically, tooth bleaching started as an in-office procedure with the use of a highly concentrated hydrogen peroxide (HP) solution requiring meticulous protection of the oral cavity by the dentist.[2] The use of a customized tray and carbamide peroxide enabled the bleaching procedure to be performed at home under the supervision of an oral health care professional.[3] Trays continue to be one of the most favorable delivery systems but were recognized to be time consuming in fabricating and also demanded significant compliance of the users. A major innovation that increased the use of over-the-counter (OTC) whitening products was the delivery of HP by strips that could be easily applied to the teeth. Thus, whitening strips (WSs) became the most popular, because of their convenience, low cost, less possible damage to the gingiva, and good esthetic results.[4]

Regardless of the bleaching technique and delivery system, the most recognized agent that causes bleaching is HP. HP when applied to the outer tooth surface readily penetrates into the tooth, interacts with stain molecules within the enamel and dentin, and may also alter the surface microscopically resulting in an increase in lightness and decrease in chroma.[5] Despite the successful use for more than 100 years, there have been concerns on the use of highly concentrated HP and its potential to induce intense inflammation in the pulp tissue.[6-8]

Additionally, the EU Council Directive 2011/84/EU stated that "Tooth whitening or bleaching products containing concentrations greater than 0.1% or less than 6% of HP are to be only sold to dental practitioners, which further promoted the search for non-peroxide whitening products and opened the market for other ingredients such as sodium hypochlorite, activated charcoal, citric acid, and phthalimido peroxy caproic acid (PAP).[9,10] Among these, sodium chlorite reacts with the citric acid and generates chlorine dioxide as an active bleaching agent.[11] Sodium hypochlorite, similar to HP in mechanism, oxidizes the double bonds within the chromogen structure.[12]

With the increase of non-peroxide whitening products on the market, there are uncertainties of their effects on enamel erosion and if the desired whitening results are indeed comparable to HP. The International Organization for Standardization (ISO) creates documents that provide requirements and guidelines to ensure that materials and products are fit for their purpose.[13] The ISO 28399 standard outlines test methods for laboratory assessment of tooth bleaching efficacy and safety requirements.[14] Based on the standard, there are no specific thresholds for bleaching efficacy, but the erosion created by a bleaching product should be less than erosion caused by 0.25% citric acid used for 4 hours. Thus, the purpose of this study was to evaluate two types of non-peroxide WSs compared to an ADA Seal of Acceptance—HP strips product in terms of whitening efficacy and enamel erosion.[15] We hypothesized that there would be no difference in efficacy and enamel erosion depth among the different experimental groups.

METHODS AND MATERIALS

Sample Selection and Preparation

Extracted sound human third molars (N=94) were collected and stored in 0.2% sodium azide solution at 4°C. Teeth were cleaned of gross debris and placed in artificial saliva at room temperature. Artificial saliva was prepared according to Shellies and others, and replaced weekly throughout the study.[16] Extracted molars were distributed into two major parts. A total of 64 teeth were used for tooth color evaluation (16 specimens/group), while another 30 teeth were used for erosion evaluation (6 specimens/group).

Experimental Groups for Color Evaluation

Prepared specimens were allocated into four groups based on severity of tooth discoloration, and bleaching

materials were used according to manufacturers' directions. NC: Negative control treated with Grade 3 water for 60 minutes; BT: Non-peroxide Brilliant Dissolving Strips (Lornamead Ltd, Wiltshire, UK), applied 5 minutes twice a day for 7 days; FM: Non-peroxide Fancymay Teeth WSs (Shenzhen Hanyun Technology Co Ltd, Shenzhen, Guangdong, China), applied 60 minutes each day for 14 days; WS: Crest 3D Brilliance HP White Strips (Procter & Gamble, Cincinnati, OH, US), applied 30 minutes each day for 16 days. To apply, WSs were removed from their liner and placed with the gel side to the buccal surface, slightly pressed against the teeth for the best contact, and the remainder folded onto the occlusal surface. Table 1 summarizes the information for the bleaching materials.

Table 1: *Summary of Whitening Materials Used*

Group	Brand Name	Listed Ingredients
NC	N/A	Water of grade 3
BT	Brilliant Dissolving Strips	Accelerator ingredients: Aqua, Sodium Chloride, Whitening Strip (WS) ingredients: PVP, Glycerin, Aqua, Citric Acid, Aroma, Polysorbate-80, Sucralose, Propylene Glycol, Cellacefate, Maltodextrin
FM	Fancymay Teeth WSs	Glycerin, Aqua, Cellulose Gum, Sodium Chlorite, Disodium EDTA, Cocos Nucifera oil, Citric Acid, and D. L-menthol
WS	Crest 3D Whitestrips Brilliance White	PVP, PEG-8, Water, HP, Acrylates Copolymer, Sodium Hydroxide, and Sodium Saccharin

Abbreviations: NC, Negative control treated with water; BT, Non-peroxide Brilliant Dissolving Strips; FM, Non-peroxide Fancymay Teeth Whitening Strips; WS, Crest 3D Brilliance Hydrogen Peroxide White Strips; HP, hydrogen peroxide.

Tooth Color Change Assessment

The step-by-step procedure from specimen preparation to instrumental color assessment is outlined in Figure 1. The roots were trimmed 3-mm apical to the cemento-enamel junction and the pulp was removed. Teeth were mounted on the top of a plastic dish with cyanoacrylate adhesive (Super Glue Liquid, 3M, St. Paul, MN) and further stabilized with acrylic resin on the lingual side. One operator performed the instrumental color measurements on the middle-third of the buccal surface using a contact type intraoral spectrophotometer (VITA Easyshade Compact, VITA GmBH, Bad Sackingen, Germany). A custom fabricated jig was used for repeated measurements on the same area. Measurements were performed under a color-controlled light box (MM 4e GTI Mini Matcher, GTI Graphic Technology, Inc, Newburgh, NY, USA) at CIE D65, a color temperature of 6500 K and light intensity of ≈1200 lux. Results were gathered by recording L^*, a^*, and b^* values at baseline (T1), 1-day posttreatment (T2), and 1-week posttreatment (T3). The overall color change, as measured with the spectrophotometer, was expressed as ΔE_{ab}^* from the Commission Internationale de l'Eclairage (CIE 1986).[17] The following equation was used and calculated relative to baseline color parameters (L^*1, a^*1, b^*1):

$$\Delta E_{ab}^* = [(L^*_2-L^*_1)^2 + (a^*_2-a^*_1)^2 + (b^*_2-b^*_1)^2]^{1/2}$$

On completion of baseline color measurements, the teeth were treated with WSs according to manufacturers' directions. The teeth were stored in artificial saliva at room temperature throughout the experimental time period.

Micro-computed Tomography (Micro-CT) for Erosion Assessment

The step-by-step procedure from specimen preparation to micro-computed tomography (micro-CT) assessment

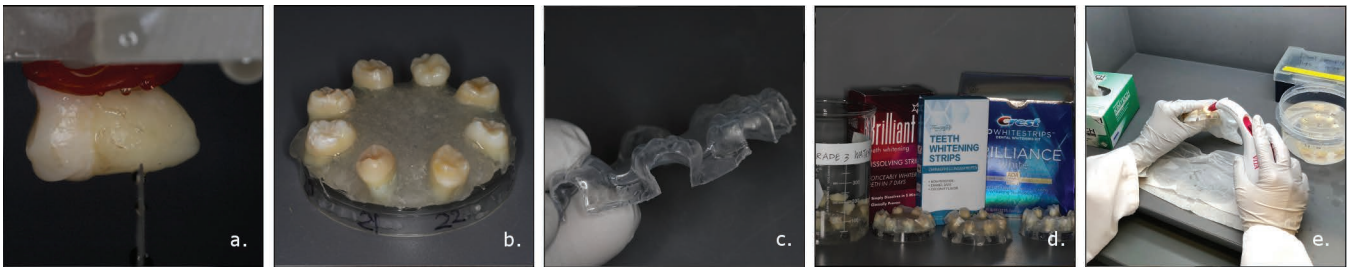

Figure 1. *Step-by-step protocol for bleaching efficacy assessment.(a) Root trimming; (b) Teeth mounting; (c) Jig fabrication; (d) Whitening treatment; (e) Instrumental color measurement.*

is outlined in Figure 2. Enamel blocks were prepared from caries-free human extracted molar teeth (N=30). Teeth were cut to a rectangular shape of 4×4×6 mm and mounted on acrylic rods with cyanoacrylate adhesive (Super Glue Liquid). The buccal enamel surfaces were polished with medium-grit paper and then sequentially polished up to P1200 paper. The flat surfaces were painted with nail-varnish (Sally Hansen, New York, NY, USA) to expose a flat 2×4-mm window. Care was taken to prevent dehydration of test specimens during the specimen preparation procedure. The specimens were randomly distributed into five groups of six specimens each. In addition to the four groups (NC, BT, FM, WS) for tooth color measurements, a positive control (PC) group was added that consisted of treatment with 0.25% citric acid (pH=3.68) for 4 hours, as per ISO 28399 guidelines.[14] Tooth whitening was performed according to manufacturers' direction on the exposed window the same way as for the color evaluation samples.

On completion of treatment, all specimens were scanned using the SkyScan 1272 desktop micro-CT system (Bruker micro-CT NV, Kontich, Belgium), with an accelerating source voltage of 100 keV, a source current of 100 mA, and an exposure time of 2600 ms. All the specimens were positioned in the same way in the center of rotation of the mounting device. During the scanning process, the samples were rotated at 180°, with an imaging voxel size of 4.5 μm and rotation step of 0.4. The images were saved as 16-bit Tagged Image File Format (TIFF) files and consequently exported to a reconstruction program (NRecon software, version 1.7.4.6; SkyScan) for the reconstruction of the 3D object. The tomographic reconstruction produced a dataset of slice views in 16 bit TIFF format, which were assessed in the analysis program (CTAn software, version 1.18.8.0; SkyScan). Figure 3 illustrates the tomographic reconstruction and digital slicing of the sample perpendicular to the occlusal surface at the middle slice. Lesion depths were assessed in the middle slice of each sample, and three vertical measurements from lesion surface to upper and bottom surface were recorded and averaged. One operator that was blinded to the treatment groups performed the reconstruction and measurements.

Statistical Analysis

G*Power 3.1.9.4 (Heinrich-Heine Dusseldorf University, Germany) was used to determine the sample size based on the following parameters: 80% power, 2.7 effect size, SD of 1.1, and four experimental groups. A minimum sample size of 16 specimens per group was assessed to be appropriate. Measurements for tooth color assessment included L^*, a^*, b^*, ΔL^*, Δb^*, and ΔE_{ab}^*. A sample size of six specimens per group were used for the erosion assessment per ISO 28399 guidelines. Kruskal-Wallis procedure was used to determine significant differences in color change and erosion depth among the groups. Dwass-Steel-Critchlow-Fligner pairwise comparisons were used when needed. Tests of hypotheses were two-sided with an alpha level of 0.05. Analysis was conducted with SAS v 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

The baseline lightness (L_1) of teeth ranged from 74.1 to 85.3, with a mean value of 79.9. Baseline chroma in the yellow-blue ranged from 16.0 to 34.2, with a mean value of 25.78. There was no statistically significant difference in any color parameter among the four groups (L_1, a_1, and b_1) at baseline ($p>0.05$, in all instances).

The overall color change (ΔE^*_{ab}) relative to baseline over time by group are summarized in Table 2 and illustrated in Figure 4 as boxplots. The magnitude of ΔE^*_{ab} was based on an increase in lightness and decrease in chroma of a^* and b^*. The mean ΔE^*_{ab} at 1-day/1-week posttreatment were 2.4/2.5, 2.8/2.9, 2.8/3.2, and 8.6/11.0 for NC, BT, FM, and WS, respectively. There was a statistically significant difference among the four groups for ΔE^*_{ab} at 1-day and 1-week posttreatment ($p<0.001$, in both instances). Group WS had the highest color change regardless of timepoint, while the other three groups did not differ from each other ($p>0.05$, for all pairwise comparisons). The "ISO/TR 28642"

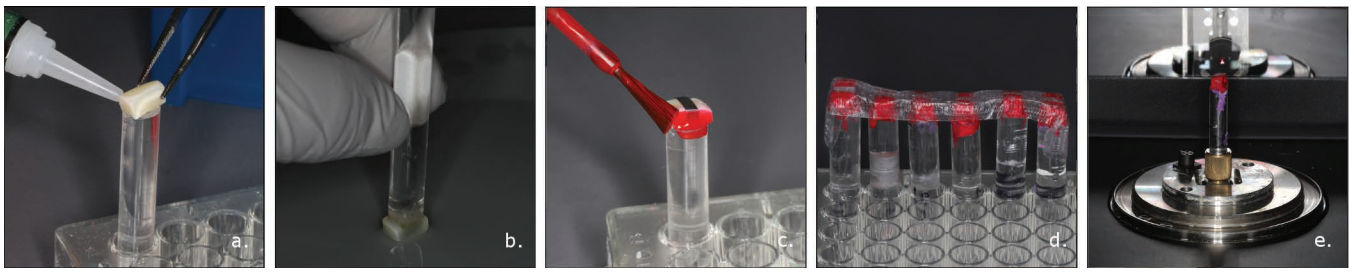

Figure 2. *Step-by-step protocol for micro-CT erosion assessment. (a) Sample mounting; (b) Sample polishing; (c) Varnish painting; (d) Whitening treatment; (e) Micro-CT scanning.*

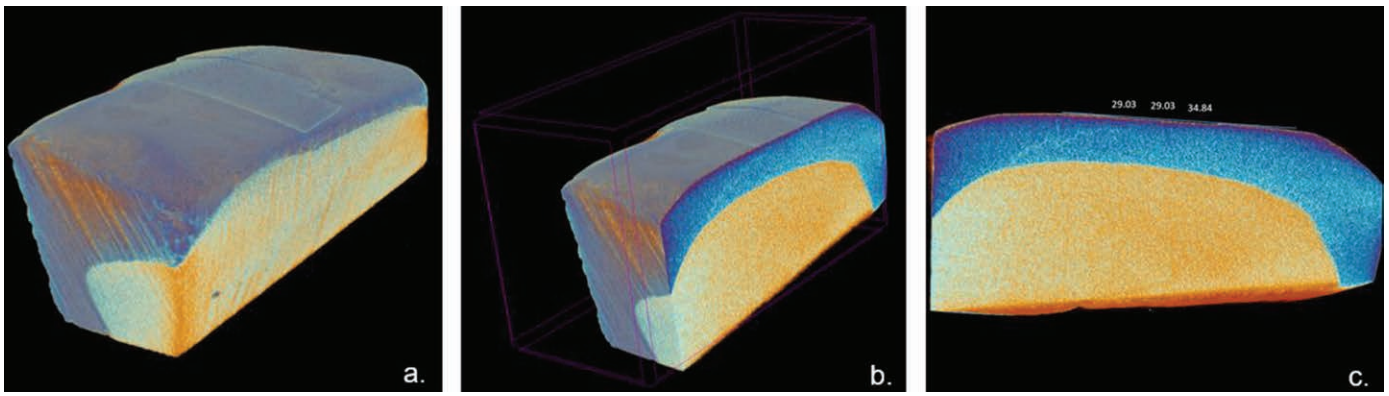

Figure 3. *Step-by-step procedure for erosion measurement. (a) 3D reconstruction of sample; (b) Digital slicing at middle slice; (c) Three vertical measurements from lesion surface to upper and bottom surface.*

Table 2. *Overall Color Change by Group Over Time (Mean±SD)*[a]

Group	NC	BT	FM	WS	*p*-value
ΔE*2-1	2.4 ± 1.3 a	2.8 ± 1.2 a	2.8 ± 1.4 a	8. 6± 1.9 b	<0.001
ΔE*3-1	2.5 ± 1.5 a	2.9 ± 1.8 a	3.2 ± 2.3 a	11.0 ± 2.9 b	<0.001

Abbreviations: NC, Negative control treated with water; BT, Non-peroxide Brilliant Dissolving Strips; FM, Non-peroxide Fancymay Teeth Whitening Strips; WS, Crest 3D Brilliance Hydrogen Peroxide White Strips.
[a] *Within rows, different lowercase letters indicate means that are statistically different after pairwise comparisons (p<0.05).*

outlines the definition of thresholds that can also be used as a reference to determine bleaching efficacy.[18] Based on the report, perceptibility threshold (PT) ΔE^*ab=1.2, is the difference in color that can be detected by 50% of observers, with the other 50% of observers noticing no difference in color between the compared objects while acceptability threshold (AT) is a difference above ΔE^*ab=2.7, where 50% of observers would consider the compared objects to be an unacceptable match. All groups exceeded the perceptibility threshold of ΔE^*_{ab}=1.2 at both timepoints.[18] Group NC was the only group that did not exceed the acceptability threshold of ΔE^*_{ab}=2.7 at both time points.[18]

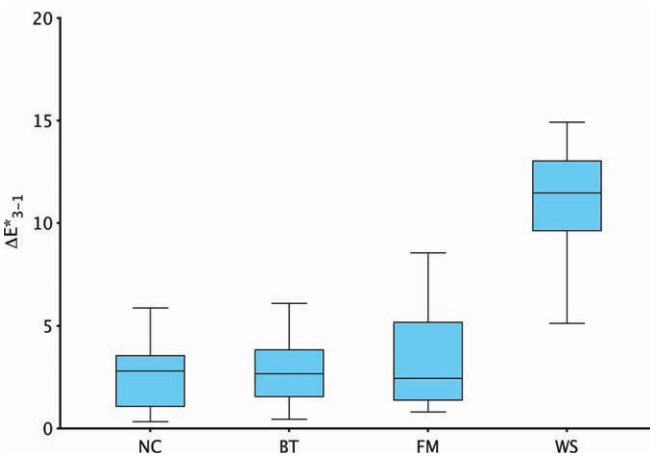

Figure 4. *Boxplots of overall color change by group at 1-week posttreatment.*

The mean erosion depths by group are summarized in Table 3 and illustrated as boxplots in Figure 5. The mean erosion depths in microns were 0.52, 0.58, 0.42, 0.49, and 29.55 for NC, BT, FM, WS, and PC, respectively. There was a statistically significant difference among the five groups (p=0.004). Group PC had the greatest erosion depth, while the other groups had negligible erosion that did not differ from each other (p>0.05, for all pairwise comparisons).

DISCUSSION

Non-peroxide dental whitening is marketed increasingly as a result of the scientific community's effort to develop innovative whitening materials. The increase is also driven by regulatory guidelines limiting the allowable concentration of HP. Manufacturers claim that such non-peroxide-based products result in instant whitening while having minimal adverse effects. However, there is scarce literature to support these claims, which leaves oral health care professionals undermined in advising the public on effective and safe whitening products.

Table 3: *Erosion Depth (μm) By Group (Mean±SD)*[a]

GROUP	NC	BT	FM	WS	PC	*p*-value
Depth	0.52 ± 0.19 a	0.58 ± 0.20 a	0.42 ± 0.16 a	0.49 ± 0.17 a	29.55 ± 3.52 b	<0.001

Abbreviations: NC, Negative control treated with water; BT, Non-peroxide Brilliant Dissolving Strips; FM, Non-peroxide Fancymay Teeth Whitening Strips; WS, Crest 3D Brilliance Hydrogen Peroxide White Strips.

[a] *Within rows, different lowercase letters indicate means that are statistically different after pairwise comparisons (*p<*0.05).*

This *in vitro* study was designed to evaluate the whitening efficacy and potential erosion of two types of non-peroxide WSs relative to a negative control and an OTC WS holding an ADA Seal of Acceptance.

Based on the results, we rejected the first null hypothesis. There was a significant difference in overall color change at 1-day and 1-week posttreatment, with the ADA Seal of Acceptance OTC whitening strip (WS) exhibiting the highest color change. The color change associated with non-peroxide WSs containing sodium chloride, sodium chlorite, and citric acid were not different from the negative control.

The negative control of water was included to add rigor to the study design, and it is important to note that the mean ΔE^*_{ab} of the negative control was close to the acceptability threshold (ΔE^*_{ab}=2.7). This is in alignment with a systematic review of *in vitro* studies that calculated an estimate of ΔE^*_{ab} = 2.9 for negative controls.[19] Thus, the tested non-peroxide WSs had comparable color change as to the use of water. The study results support the findings by Kielbassa and others that reported negligible color change with non-peroxide whitening kits.[11] However, our results are in discordance with a study that used the same product "Brilliant 5-minute kit" and reported a significant color change with the non-peroxide OTC product.[10] There may be several reasons for the discrepancy. The Cohen and others study pretreated extracted teeth with a staining solution, measured tooth color visually with a shade guide, and used a small number of teeth for each group.

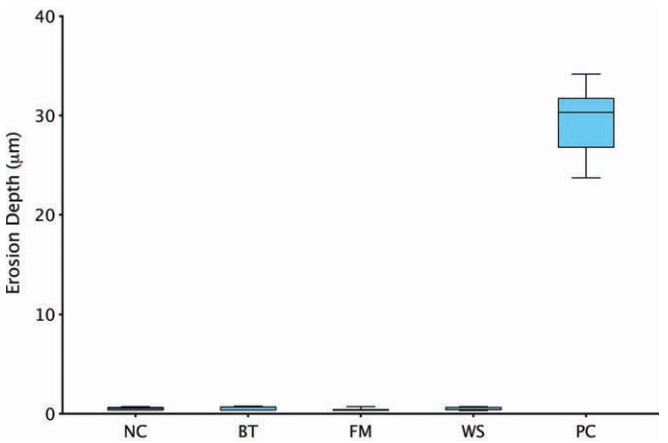

Figure 5. *Boxplots of erosion depths by group.*

The mean ΔE^*_{ab} = 11.0 of the Crest Brilliance WS in our study was comparable to another study that evaluated a similar Crest WS and reported a mean ΔE^*_{ab} = 10.0 at 1-week posttreatment.[20] The efficacy results of the current study prompted the need to evaluate the mechanism of non-peroxide whitening agents. The mechanism of HP has been extensively studied and is based on well-documented diffusion of HP into the tooth structure that reaches the pulp within 5-15 minutes.[21] During the course of diffusion, the active oxygen radicals interact with the chromophores, resulting in lightening of the tooth.[5] Thus, further studies on the mode of action of non-peroxide whitening agents to support their efficacy are warranted.

There is no dispute that the two most commonly reported side effects of tooth whitening are tooth sensitivity and gingival irritation.[22] With the increased use of OTC products that are not supervised by oral heath professionals, there have also been concerns about tooth structure integrity such as changes to surface roughness, microhardness, and loss of dental hard tissues associated with an acid attack not from bacterial origin, which is defined as erosion.[14,23-27]

Based on the results, we rejected our second null hypothesis. There was a difference among the tested groups with the positive control of 0.25% citric acid for 4 hours, showing an erosion depth of approximately 30 microns. This is comparable to another study that used 1.0% citric acid for 1 hour and reported enamel loss of 24 microns.[28] All tested WSs, regardless of composition, had negligible erosion, which was comparable to the use of water. This is in agreement with a study that found that whitening did not increase the susceptibility of enamel to erosion.[25] To our best knowledge, this is the first study that compared erosion depth of non-peroxide versus peroxide WSs using micro-CT. Micro-CT was used to enable 3D reconstruction of the samples and digitally slicing the samples for measurements. The results are significant in informing users that OTC products, when used according to manufacturer's directions they do not cause potential erosion to the enamel. The limitations of this study include the lack to fully replicate the dynamics of the *in vivo* oral environment and not

evaluating whether erosion may have been detected with potential overuse of the products.

CONCLUSIONS

The study evaluated the efficacy and safety of non-peroxide strips compared to HP WSs. Within the limitations of the study, we conclude that peroxide WSs had superior whitening efficacy compared to non-peroxide strips. None of the tested products compromised tooth structure integrity by potential enamel erosion. Future studies should further evaluate other aspects of safety such as changes in microhardness, surface roughness, and diffusion into the tooth. Additionally, the effect of adaptation of various strip systems relative to color change and adverse effects could be explored.

Acknowledgement

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Regulatory Statement

The use of extracted teeth without identifiers was determined to be nonhuman subject research by the local human subjects oversight committee.

Conflict of Interest

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

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Longitudinal *In Vitro* Effects of Silver Diamine Fluoride on Early Enamel Caries Lesions

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Clinical Relevance

Fluoride varnish is likely a better choice than silver diamine fluoride (SDF) for treatment of early, incipient, noncavitated, white-spot enamel lesions.

SUMMARY

This laboratory study evaluated the longitudinal surface microhardness changes in early, incipient, noncavitated, white-spot, enamel caries lesions treated with silver diamine fluoride (SDF). Five intervention groups (SDF, $AgNO_3$, KF, 5% sodium fluoride varnish (FV), deionized water (DI)) × two-time intervals after intervention (immediate & delayed pH-cycling) resulted in 10 groups (n=18). Silver nitrate ($AgNO_3$) and potassium fluoride (KF) groups served as controls to assist in evaluating if remineralization effects were due to the silver or fluoride component in SDF. Early, incipient, noncavitated, white-spot, enamel caries lesions were created in bovine enamel, the extent of demineralization was determined using Vickers surface microhardness (VHN_{lesion}). Intervention treatments were applied. Half the specimens from each group underwent immediate 5-day pH-cycling, and half were stored in an incubator with artificial saliva for 2 weeks before undergoing 5-day pH-cycling. After pH-cycling, lesion hardness was evaluated using VHN_{post}. Specimens were then exposed to a second demineralization challenge, and lesion softening was evaluated (VHN_{secdem}). Hardness variables were calculated: $\Delta VHN = VHN_{post} - VHN_{lesion}$; $\Delta VHN_{secdem} = VHN_{secdem} - VHN_{post}$. Data were analyzed using two-way ANOVA (α=0.05). Immediately cycled, SDF had significantly (p<0.0001) greater remineralization than DI, $AgNO_3$, and FV. All delayed cycling groups had significantly greater remineralization than FV (p<0.0001). Significantly greater remineralization was noted in delayed $AgNO_3$

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($p \leq 0.0001$), DI ($p=0.0003$), and FV ($p=0.0006$) compared to immediately cycled. After the second demineralization challenge, FV had significantly less surface softening than $AgNO_3$ ($p=0.0002$), DI ($p=0.0003$), KF ($p=0.0225$), and SDF ($p=0.0388$) intervention groups. No significant difference was found between the pH-cycle timings ($p=0.2710$). Based the present findings, FV may be better suited than SDF to treat early, incipient, noncavitated, white-spot, enamel caries lesions.

INTRODUCTION

Silver diamine fluoride (SDF) has been used for over 80 years in Japan, Argentina, Australia, Brazil, and China,[1,2] as a topical medicine at concentrations of up to 38% for the treatment of tooth hypersensitivity and to arrest caries lesions.[2] In 2014, the use of 38% SDF in the United States was approved by the Food and Drug Administration (FDA) for treating adults with dentin hypersensitivity.[2,3,4,5] In 2018, the American Dental Association (ADA) approved use of SDF as an interim caries-arresting medicament.[2] A recent review indicated that 38% SDF could be used to treat asymptomatic cavitated coronal caries as well as cavitated and noncavitated root caries.[3] While both SDF and fluoride varnish (FV) have been approved by the FDA for treatment of tooth sensitivity, only FV is currently used off-label for primary prevention of dental caries lesions.[2] The 2018 clinical practice guidelines published by the ADA recommended biannual applications of 38% SDF as the treatment of choice to arrest cavitated lesions on coronal surfaces of both primary and permanent teeth.[3] However, the ADA publication also stated that there currently is not enough research to recommend using SDF on noncavitated proximal lesions.[3]

In vitro studies have shown that SDF can penetrate up to 25 microns into enamel and up to 200 microns into dentin, which leads to two to three times more fluoride retention compared to applications of sodium fluoride (NaF) or stannous fluoride (SnF_2).[2,6,7] This greater retention of fluoride suggests SDF will have a greater efficacy in preventing and arresting caries lesions than NaF or SnF_2.[7] It was also reported in a systematic review and meta-analysis that SDF was 89% more effective than other fluoride treatments in arresting and controlling caries.[4] Similarly, *in vitro* studies found that upon a second demineralization challenge, demineralized dentin treated with SDF was better able to resist further demineralization compared to nondemineralized dentin.[8] This increased resistance to further demineralization was attributed to both the high fluoride levels (44,800 ppm) as well as the high levels of silver that were deposited on the demineralized surfaces after application of SDF.[4,8]

A review article suggested that SDF was more effective than FV and could be a possible caries-prevention intervention, but further research was needed.[7] Additionally, the University of California San Francisco (UCSF) published protocol for caries arrest using SDF noted that annual application of SDF produced better prevention of caries lesions in both children and elderly compared to applying FV four times a year.[2] In one of the few clinical studies that examined SDF efficacy in preventing caries in the permanent dentition, it was found that SDF was 65% effective in preventing caries in permanent first molars.[9] Application of SDF only takes one minute[2] and has shown to reduce the progression of caries by 89%.[4] However, research is lacking on the effects of SDF on early enamel caries lesions and its longitudinal effects.[10,11] Likewise, the acid resistance of remineralized enamel lesions that have been previously treated with SDF has not yet been investigated. Therefore, the purpose of our research was to investigate the longitudinal surface microhardness changes in early, incipient, noncavitated white-spot, enamel caries lesions treated with SDF. This *in vitro* study aimed to test the hypotheses that: 1) SDF treatment will result in increased surface microhardness of early, incipient, noncavitated, white-spot lesions in enamel compared to all other tested interventions; and 2) specimen storage for 2-weeks in artificial saliva will result in greater surface rehardening in lesions treated with SDF compared to all other interventions.

METHODS AND MATERIALS

Specimen Preparation

Enamel specimens, 4 × 4 mm, were obtained from bovine teeth using a low-speed saw (IsoMet, Buehler, Lake Bluff, IL, USA). During specimen preparation, all specimens were stored in deionized water (DI) containing 0.1% thymol. A polishing unit (Struers Rotopol 31/Rotoforce 4, Struers Inc, Cleveland, OH, USA) was used to grind and polish specimens to create flat, smooth, and uniform enamel and dentin surfaces for microhardness testing. The enamel surface of the specimens was ground smooth with 1200-, 2400-, and then 4000-grit silicon carbide paper, followed by a 1-μm diamond polishing suspension on a polishing cloth. After polishing, specimens were rinsed and sonicated in DI for 3 minutes as a final cleaning step. Under 20× magnification, specimens were inspected for cracks, hypomineralization (white spots) areas, and any other flaws present in the enamel surface, which would result in their exclusion from use in this study. All surfaces of

specimens, except the enamel surface to receive testing, were coated with acid-resistant, colored nail varnish (Sally Hansen Advanced Hard As Nails Nail Polish, Red, New York, NY, USA). Prepared specimens were stored at 100% relative humidity at 4°C until further use. A total of 219 specimens were prepared.

Sound Enamel Surface Microhardness

Using a Vickers diamond indenter with a 200-g load for 10 seconds, four baseline indentations (2100 HT; Wilson Instruments, Norwood, MA, USA) were placed 150 μm to the right of the center of each sound enamel specimen (approximately 150 μm apart from each other). The average sound Vickers hardness values (VHN_{sound}) were recorded for each specimen. Specimens with a VHN_{sound} between 300 and 400 were included in the study.

Artificial Caries Lesion Creation

Artificial caries lesions were created in the specimens by a 36-hour immersion in a solution of 0.1 M lactic acid, 0.2% Carbopol 907, 3.0 mM $CaCl_2 \times 2H_2O$, 6.0 mM KH_2PO_4, 63.0 mM KCl, and 3.1 mM NaN_3, with the pH adjusted to 5.0 using KOH. Upon removal from the chemical lesion creation solution, specimens were rinsed with DI.

Postlesion Creation Surface Microhardness

Postlesion creation indentations (VHN_{lesion}) were placed approximately 150 μm to the right of the VHN_{sound} indentations, as described when obtaining sound microhardness values. Specimens were included if their mean VHN_{lesion} value fell within the range of the mean VHN_{lesion} value of all specimens +/− 2 standard deviations of VHN_{lesion} of all specimens and had a standard deviation less than 12.5. The percentage surface microhardness change ($\%SMHC_{lesion}$) was calculated as follows: $\%SMHC_{lesion} = (VHN_{lesion} - VHN_{sound})/VHN_{sound} \times 100\%$. The 180 accepted specimens were stratified into 10 groups (two pH cycle timings × five intervention groups; n=18 per group) to ensure no statistically significant differences in mean VHN_{lesion} between the groups.

Application of Interventions

The five intervention groups with their active ingredients are displayed in Table 1. FV was applied according to the manufacturer's instructions. Specimens were air dried for 1 minute after application and then rinsed with DI. The applied FV was not artificially removed from the specimens. For all other interventions, the UCSF SDF application protocol was followed.[2,12] A microbrush was used to apply the intervention for 10 seconds to a dried enamel surface. After application, specimens were air dried for 1 minute and then rinsed with DI.[2]

pH Cycling Phase

The chosen pH cycling model was based on a model that is described elsewhere.[13,14] Immediately following application of the interventions, half of the specimens for each group (n=18) underwent immediate pH cycling for 5 days. The remaining specimens were stored for 2 weeks to be subjected later to the same pH cycling procedure.

The daily pH cycling schedule (Table 2) included two 1-minute fluoride exposures separated by four alternating cycles of 30-minute remineralization in artificial saliva [1.5 mM calcium dichloride ($CaCl_2$) × 2 H_2O (water); 0.9 mM KH_2PO_4 (potassium phosphate); 130.0 mM KCl

Table 1: *Intervention Group Products*

Intervention	Study Purpose	Manufacturer	Fluoride Source and Concentration	Silver Concentration	Noteworthy Ingredients
Silver Diamine Fluoride (SDF)		Elevate Oral Care	38% SDF; 44,800 ppm	253,900 ppm	—
Prevident 5% NaF Varnish (FV)	Clinical Reference Standard	Colgate	5% NaF; 22,600 ppm	—	Xylitol
Potassium Fluoride (KF)	Fluoride Control	Sigma-Aldrich	44,800 ppm	—	—
Silver Nitrate ($AgNO_3$)	Silver Control	Sigma-Aldrich	—	253,900 ppm	—
Deionized Water (DI)	Negative Control	—	—	—	—

Table 2: *Daily pH Cycling Regimen*

Duration	Specimen Treatment
1 minute	Fluoride Toothpaste Exposure
30 minute	Remineralization
60 minute	Demineralization
30 minute	Remineralization
60 minute	Demineralization
30 minute	Remineralization
60 minute	Demineralization
30 minute	Remineralization
60 minute	Demineralization
1 minute	Fluoride Toothpaste Exposure
(Overnight)	Remineralization

(potassium chloride); 20.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); 3.1 mM NaN_3 (sodium azide), adjusted to pH 7.0 with KOH (potassium hydroxide)],[12] followed by 60-minute demineralization in the lesion creation solution. The specimens were stored in artificial saliva overnight.

For the fluoride exposures, a fluoride toothpaste slurry was prepared by mixing toothpaste (Crest Cavity Protection, 0.243% sodium fluoride; Proctor and Gamble, Mason, OH, USA) with artificial saliva in a 1:2 ratio in a beaker with a magnetic stirrer.[15] Fresh slurry was prepared immediately prior to each 1-minute fluoride exposure.

The specimens that underwent the 2-week delayed pH cycling were stored in an incubator at 37°C in artificial saliva at approximately 100% relative humidity. During the 2 weeks of storage, specimens were taken out of the incubator daily, rinsed with DI, blotted dry, and a new 50-μL drop of artificial saliva was pipetted on the top of specimens. After 2 weeks of storage, the delayed specimens followed the 5-day pH cycling described above.

Post-pH Cycling Surface Microhardness

Post-pH cycling indentations (VHN_{post}) were placed approximately 150 μm to the left of the VHN_{sound} indentations, as described when obtaining sound microhardness values. The extent of surface rehardening was calculated as follows: $\Delta VHN = VHN_{post} - VHN_{lesion}$. The $\%SMHC_{post}$ was calculated as follows: $\%SMHC_{post} = (VHN_{post} - VHN_{lesion})/VHN_{lesion} \times 100\%$.

Secondary Demineralization

After post-pH cycling microhardness evaluations, all specimens were immersed for 24 hours in the chemical lesion creation solution, as described above. Subsequently, specimens were rinsed with DI.

Postsecondary Demineralization Surface Microhardness

Postsecondary demineralization indentations (VHN_{secdem}) were placed approximately 150 μm to the left of the VHN_{post} indentations, as described when obtaining sound microhardness values. The extent of surface softening was calculated as follows: $\Delta VHN_{secdem} = VHN_{secdem} - VHN_{post}$. The %SMHC was calculated as follows: $\%SMHC_{secdem} = (VHN_{secdem} - VHN_{post})/VHN_{post} \times 100\%$. Positive ΔVHN and %SMHC values indicated lesion rehardening, while negative values indicated further demineralization.

Surface Images

Surface images of all the specimens were acquired after completion of the final hardness measurements. For this, one representative specimen from each of the 10 groups was placed under an optical microscope (D3100, Nikon, Tokyo, Japan) equipped with a digital camera (Infinity 1, Lumenera, Ottawa, ON, Canada). One image of each specimen was acquired under standardized conditions and saved.

Radiographs

Four specimens from each of the 10 groups, which had representative ΔVHN_{secdem} data for their group were selected for radiographic analysis. The 40 specimens were mounted on plastic rods and sectioned with a hard tissue microtome (Silverstone-Taylor Hard Tissue Microtome, Series 1000 Deluxe; Silverstone-Taylor, SciFab, Lafayette, CO, USA). One approximately 100-μm section was obtained from each specimen. The sections were X-rayed at 45 kV and 45 mA at a fixed distance for 12 seconds.

Statistical Analysis

Outcomes of the primary variables, ΔVHN and ΔVHN_{secdem}, were analyzed using two-way ANOVA, with factors for interventions ($AgNO_3$, DI, FV, KF, and SDF) and pH cycling modes (two-week delay and immediately), as well as interactions between the factors to identify the significant effects of intervention and pH cycling. All pair-wise comparisons from ANOVA analysis were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5%. Summary statistics were calculated for the exploratory objectives $\%SMHC_{lesion}$, $\%SMHC_{post}$, and $\%SMHC_{secdem}$. Analysis was performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Sample Size Calculations

With a sample size of 18 specimens per treatment-storage combination, the study had 80% power to detect a ΔVHN post-pH cycling difference of 14.6 between any two groups, assuming two-sided tests, each conducted at a 5% significance level and ΔVHN standard deviation 15.

RESULTS

VHN_{sound} (mean ± standard deviation) varied between groups from 363 ± 18 to 378 ± 13 (Table 3). VHN_{lesion} was virtually identical between the groups (all 81±8). Lesion creation resulted in an approximate 78% reduction in mean hardness in all the groups ($SMHC_{lesion}$). The interaction between interventions and pH cycling modes was significant for ΔVHN ($p<0.0001$) but not for ΔVHN_{secdem} ($p=0.8636$). The ΔVHN_{secdem} data were affected by the type of intervention ($p=0.0012$) only and not by pH cycling modes ($p=0.2710$). The ΔVHN data are shown in Figure 1, whereas the ΔVHN_{secdem} data can be found in Figure 2. All other hardness data can be found in Table 3.

Rehardening

Rehardening (ΔVHN; mean ± standard deviation; Figure 1) values for specimens that were immediately pH-cycled were significantly ($p<0.0001$) higher in the SDF (62±14) and KF (64±18) intervention groups than all other intervention groups, which ranged between 9±10 (FV) and 43±18 ($AgNO_3$). There was no statistically significant difference between KF and SDF groups ($p=0.6947$) and between $AgNO_3$ and DI (40±17) treated specimens ($p=0.5132$), respectively. However, both $AgNO_3$ and DI intervention groups had significantly ($p<0.0001$) greater rehardening than the FV intervention specimens.

In the 2-week delayed pH-cycled specimens, SDF (60±14), KF (62±14), $AgNO_3$ (69±15), and DI (57±13) groups had significantly ($p<0.0001$) greater rehardening values than the FV (26±11) group (Figure 1). While the $AgNO_3$, DI, and FV intervention groups all experienced significant increases in rehardening values after the 2-week delay, only the $AgNO_3$ intervention group had statistically ($p=0.0127$) greater rehardening values than the DI intervention group. However, no statistically significant difference was found between the rehardening values of $AgNO_3$ and KF ($p=0.1255$), $AgNO_3$ and SDF ($p=0.0556$), DI and KF ($p=0.3285$), DI and SDF ($p=0.5542$), or KF and SDF ($p=0.6991$) intervention groups.

When comparing rehardening (ΔVHN) values of each intervention group to the different pH-cycles, a substantial and significant increase in rehardening was noted amongst the $AgNO_3$ ($p<0.0001$), DI ($p=0.0003$), and FV ($p=0.0006$) intervention groups after the 2-week delay compared to their respective immediately cycled rehardening values (Figure 1). The opposite effect, though not statistically significant, was seen in both the KF ($p=0.6301$) and SDF ($p=0.6343$) intervention groups, with greater rehardening values recorded in immediately pH-cycled specimens compared to the 2-week delayed pH-cycled specimens (Figure 1).

The $\%SMHC_{post}$ data (Table 3) mirrored the ΔVHN data (Figure 1) in that it showed the same rank order of rehardening for all the interventions and both the models.

Table 3: *Vickers Hardness Data and Calculated Variables for All Interventions (Mean [SD])*

Intervention	Mode	VHN_{sound}	VHN_{lesion}	$\%SMHC_{lesion}$	VHN_{post}	$\%SMHC_{post}$	VHN_{secdem}[a]	$\%SMHC_{secdem}$[a]
SDF	Immediate	374 (14)	81 (8)	−78 (2)	143 (17)	77 (18)	134 (15)	−6 (10)
FV		364 (15)	81 (8)	−78 (2)	90 (15)	11 (12)	91 (24)	3 (35)
KF		371 (26)	81 (8)	−78 (2)	145 (23)	79 (19)	133 (20)	−8 (9)
$AgNO_3$		363 (18)	81 (8)	−78 (3)	124 (16)	54 (14)	111 (12)	−11 (6)
DI		370 (17)	81 (8)	−78 (2)	121 (20)	50 (21)	106 (13)	−12 (7)
SDF	Delayed	364 (14)	81 (8)	−78 (2)	141 (16)	75 (18)	130 (17)	−7 (14)
FV		372 (14)	81 (8)	−78 (2)	107 (14)	32 (14)	102 (28)	−4 (23)
KF		378 (13)	81 (8)	−79 (2)	143 (18)	77 (17)	132 (20)	−7 (8)
$AgNO_3$		366 (18)	81 (8)	−78 (3)	150 (16)	87 (23)	130 (18)	−13 (8)
DI		369 (15)	81 (8)	−78 (2)	138 (17)	71 (16)	122 (19)	−12 (9)

Abbreviations: VHN, Vickers hardness number of sound enamel (VHN_{sound}), after lesion creation (VHN_{lesion}) or after completion of the pH cycling phase (VHN_{post}); %SMHC, percent surface microhardness change after lesion creation ($\%SMHC_{lesion}$), after completion of the pH cycling phase ($\%SMHC_{post}$) or after secondary demineralization ($\%SMHC_{secdem}$); SDF, silver diamine fluoride; FV, fluoride varnish; KF, potassium fluoride; $AgNO_3$, silver nitrate; DI, deionized water.

[a]*Individual group means are shown for information only; see results and Figure 2 for intervention means for $\%SMHC_{secdem}$.*

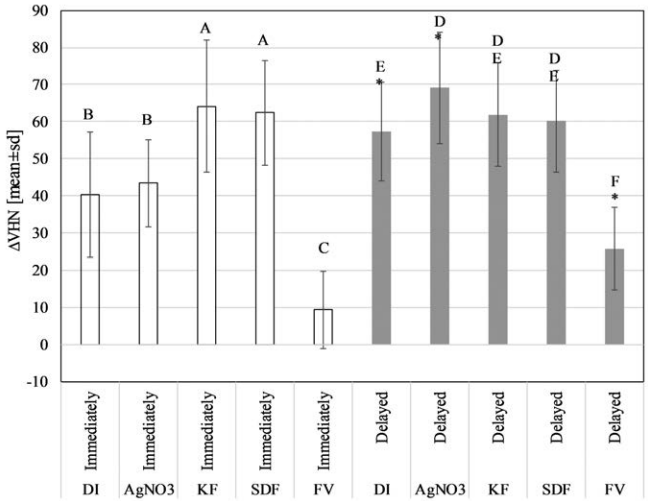

Figure 1. *ΔVHN Data Summary Chart. Mean change in surface microhardness (ΔVHN = VHN_{post} - VHN_{lesion}). Significant differences between intervention groups of the same pH cycle timing are represented by different letters. Asterisks indicate a significant difference compared to immediately cycled counterparts. Abbreviations: ΔVHN, mean change in Vickers hardness number; VHN_{post}, Vickers hardness number after completion of the pH cycling phase; VHN_{lesion}, Vickers hardness number after lesion creation; DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

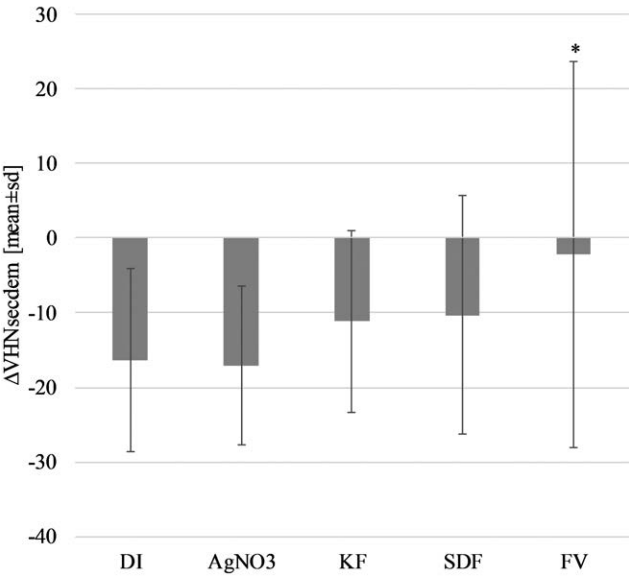

Figure 2. *ΔVHNsecdem Data Summary Chart. Mean change in surface microhardness (ΔVHNsecdem = VHNsecdem - VHNpost). Asterisk indicates FV intervention significantly different than all other interventions. Abbreviations: ΔVHN_{secdem}, mean change in Vickers hardness number after secondary demineralization; VHN_{post}, Vickers hardness number after completion of the pH cycling phase; DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

Secondary Demineralization

Specimens in the FV intervention group (ΔVHN_{secdem}; mean ± standard deviation: −2±26) had significantly less surface softening than specimens in the $AgNO_3$ (−17±11; p=0.0002), DI (−16±12; p=0.0003), KF (−11±12; p=0.0225), and SDF (−10±16; p=0.0388) intervention groups (Figure 2). However, no statistically significant difference was found between the surface softening values of DI and $AgNO_3$ (p=0.8562), DI and KF (p=0.1785), DI and SDF (p=0.1178), $AgNO_3$ and KF (p=0.1273), $AgNO_3$ and SDF (p=0.0813), or KF and SDF (p=0.8254) intervention groups.

The $\%SMHC_{secdem}$ data mirrored the ΔVHN_{secdem} data (Figure 2) in that it showed the same rank order of softening for all the interventions and both the models (FV: −1±29; $AgNO_3$: −12±7; DI: −12±8; KF: −7±8; and SDF: −7±12).

Surface Images

Figure 3 shows surface images of a representative specimen from each intervention group and model. Specimens in the FV, KF, and DI groups displayed a more or less natural tooth color, irrespective of the model. However, dark staining can be seen in both SDF and $AgNO_3$ groups. Comparing between models, it appears that specimens in the SDF groups were less dark in the delayed than in the immediate model, whereas the opposite was the case in the $AgNO_3$ groups.

Radiographs

Figure 4 shows a cross-sectional radiographic image of a representative specimens from each group that was obtained after secondary demineralization. An early subsurface caries lesion of slightly varying severity can be observed in all the specimens.

DISCUSSION

Caries management has shifted from surgical treatment to a more preventive approach, focusing on detecting and arresting caries lesions at early stages.[16,17] This early detection and prevention trend has led to further research into the different applications of fluoride, such as SDF, and resistance to subsequent acid attacks after treatment with these atraumatic, noninvasive, and prevention modalities. However, research lacks on the effects of SDF on early enamel caries lesions and the acid resistance of remineralized enamel lesions previously treated with SDF.[10,11]

The purpose of immediate pH cycling was an attempt to mimic the effects of a patient with poor oral hygiene that undergoes a second demineralization immediately

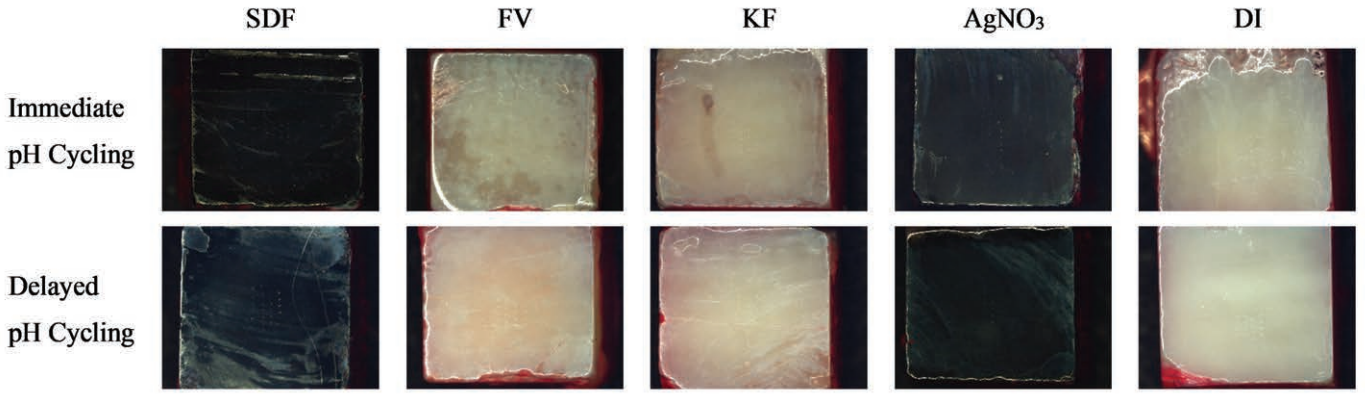

Figure 3. *Surface images of one representative specimen from each intervention group and model obtained after secondary demineralization. Abbreviations: DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

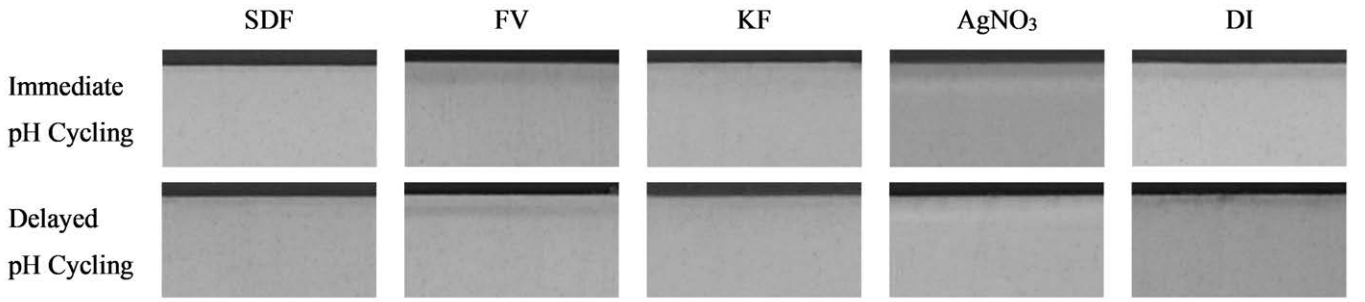

Figure 4. *Radiographic images (500 µm width) of a cross-section of one representative specimen from each intervention group and model obtained after secondary demineralization. Abbreviations: DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

after application of SDF. The purpose of the 2-week delayed storage in artificial saliva before being pH cycled for 5 days was to mimic a patient with good oral hygiene that reverts to poor oral hygiene habits and is then exposed to a second demineralization 2 weeks after application of SDF.

Vickers SMH was chosen over the "gold standard" TMR to analyze the changes in surface hardness, as Vickers is better equipped to analyze shallow lesions.[12,18-20] Utilization of V-SMH also allowed us to analyze changes in specimen hardness throughout different stages of our study, as TMR is inherently destructive.1[2,21] We did, however, include one radiographic image of a representative specimen from each group (Figure 4) for better visualization. By analyzing surface rehardening and softening with V-SMH, it provided a more clinically similar evaluation of our lesions, as practitioners often diagnose caries lesion arrest by the hardness of the lesion surface along with its appearance.[22] Furthermore, percentage changes in surface hardness were calculated at all stages of the experiment (Table 3) to allow for better interpretation of the present findings and their possible extrapolation to the clinical situation.

Based on our results, our first hypothesis that SDF treatment would result in increased surface microhardness of early, incipient, noncavitated, white-spot lesions in enamel compared to all other tested interventions, can be partially accepted, as SDF and KF treatments resulted in similar extents of surface microhardness recovery. Both SDF and KF intervention groups had the same amount of fluoride (44,800 ppm fluoride), which can explain these results and also highlight that silver ions in SDF do not appear to interfere in the remineralization process. The second hypothesis that specimen storage for 2 weeks in artificial saliva would result in greater surface rehardening in lesions treated with SDF compared to all other interventions, however, was rejected. This is speculated to be due to the low viscosity of SDF, which appeared to provide a more immediate impact on surface rehardening compared to the more viscous FV that remained on the enamel surface longer and resulted in greater remineralization of the delayed FV group compared to its immediate counterpart, unlike what the SDF group experienced.

Results for $\Delta VHN_{postlesion}$ data representing the extent of surface rehardening were mostly as anticipated.

Both the SDF and KF intervention groups exhibited the greatest fluoride concentration, which also resulted in the greatest amount of surface rehardening. Similar findings were reported in other studies.[6,23,24] While the $AgNO_3$ group did not contain fluoride, the low solubility of silver ions has been found to play a role in increasing caries lesion hardness.[25] We, therefore, anticipated the $AgNO_3$ group to exhibit some rehardening, but the effect of the silver ions may have been diluted by the twice daily fluoride exposures, thus limiting the full effects of the silver ions.

The post-pH cycling data (Figure 1) was surprising in that all the intervention groups, including DI, had statistically significant greater surface rehardening than FV. This could be due to the fact that the FV remained on the specimens prior to undergoing pH cycling, which is something that has not been done before. The purpose in removing FV prior to further testing is to represent the FV film being mechanically removed in the oral cavity as would be experienced with toothbrushing and mastication.[6,15,25,26,28] However, the present study aimed to mimic FV being retained in hard-to-reach areas, such as interproximal or occlusal areas, as well as buccal surfaces of partially erupted third molars. The FV film may have provided protection as a mechanical barrier limiting the full effects of the pH cycling regimen, thus affecting the FV hardness data. It is also important to note that the FV film appeared to have naturally worn off the specimens during the pH cycling phase, which enabled V-SMH testing. While the FV treated specimens did not experience remineralization from fluoride, calcium and phosphate ions diffusing through the varnish coating, this could occur in some FV as different varnishes have different permeability characteristics. This may be an area worth exploring in future studies.

The high variability we encountered in both the FV groups after secondary demineralization is likely due to the continuous wearing off of the FV throughout the study. While it did not affect hardness measurements directly, it may have done indirectly, as a thin film of resin may have remained in certain areas on the specimens' surface, thus protecting some areas from an acid attack where still present and offering no protection for other specimens where it already wore off. This scenario also likely occurs *in vivo*, and future research may consider studying differences in wear resistance between different FVs.

When evaluating the ΔVHN data between the different pH-cycle timings, it is apparent that the $AgNO_3$, DI, and FV intervention groups had significantly greater surface rehardening after the 2-week delay compared to immediately cycled specimens (Figure 1). The results for the $AgNO_3$ group could be explained by the possibility that silver ions require more time to interact with enamel and enhance remineralization, as speculated in a similar study.[6] Interestingly, both immediately cycled KF and SDF groups experienced significantly greater surface rehardening than immediately cycled $AgNO_3$; but after the 2-week delay, the $AgNO_3$ group had the numerically greatest extent of surface rehardening of all the groups. It can be speculated that fluoride in KF and SDF provides a more immediate impact on surface rehardening, while silver ions in $AgNO_3$ assist with sustaining surface hardness over time. We were also intrigued to find that the 2-week delayed SDF did not exhibit greater surface rehardening than each of its individual components—silver and fluoride, which were represented by the $AgNO_3$ and KF intervention groups.

Additionally, the low viscosity of the SDF, $AgNO_3$, and KF interventions may have provided an advantage over the FV group. It has been noted in other studies that the viscosity of products may play a role in fluoride absorption to enamel surfaces.[27] The lower the viscosity, the greater the amount of surface rehardening may be experienced, such as what we saw with our SDF, $AgNO_3$, and KF groups, due to the liquid being able to fully contact the enamel surface and perhaps also penetrate deeper into the caries lesion, and thus resulting in better fluoride ion absorption.[28] On the contrary, the more viscous a product is, such as our FV treatment, the more it may result in a slower diffusion of fluoride ions into the enamel surface as well as a prolonged contact time.[28]

The secondary demineralization data, ΔVHN_{secdem} data (Figure 2), revealed no significant differences between the different pH-cycle timings. However, all intervention groups exhibited significantly more surface softening compared to the FV intervention group. This was surprising, especially since SDF contained almost twice the amount of fluoride as FV. However, these findings are in agreement with previous studies, which found SDF provided a short-term increase in surface microhardness of demineralized enamel but was not as effective as FV in reducing enamel surface demineralization.[27,28] It was also found that while fluoride in SDF reduced surface softening in enamel, it was not able to prevent subsurface mineral loss to the same extent as FV.[27] This difference between SDF and FV was speculated to be due to SDF forming and releasing a smaller amount of fluoride on the enamel surface than FV.[27] Another possible reason for this finding was that silver in SDF may have competed with fluoride depositing on the enamel surface, thus making SDF less effective in preventing further surface softening on enamel compared to FV.[27]

Interestingly, there was no significant difference in the extent of surface softening after secondary demineralization between DI, $AgNO_3$, KF, or SDF (Figure 2). This finding correlates to what other research concluded, that silver ions found in $AgNO_3$ and SDF produce minimal effects on the prevention of enamel demineralization, explaining why $AgNO_3$ and DI had no significant difference in amount of surface softening.[29] In another study, it was speculated that SDF was better at preventing demineralization in dentin than in enamel due to the silver in SDF expressing a greater affinity to bind to proteins found in dentin, which are absent from enamel.[27] Research has also found SDF to not be as effective as FV in preventing demineralization of enamel upon exposure to a demineralization challenge, which correlates with our ΔVHN_{secdem} findings.[27] However, we expected no significant differences to exist between $AgNO_3$, KF, or SDF, given the synergistic effects of the silver and fluoride components in SDF. Additionally, we expected KF and SDF to have significantly less surface softening than DI, due to the high concentration of fluoride, which has shown to inhibit enamel demineralization.[6,24,25,30]

While we did not quantify enamel discoloration as a result of an SDF treatment presently, it is apparent that a single application of SDF to an early enamel caries lesion will result in dark staining that also appears to be resistant to repeated acid challenges (Figure 3). A similar observation was made in the $AgNO_3$-treated specimens, however, not in the other groups, suggesting that the staining is due to silver rather than fluoride ions.

Several limitations need to be considered in the interpretation of the present findings. A chemically induced (artificial) enamel caries lesions was employed. While universally accepted in *in vitro* and *in situ* caries research, these lesions are only a surrogate for *in vivo* lesions. In the oral cavity, caries lesions form over considerably longer periods of time, including periods of remineralization, exposure to fluoride, and salivary proteins. This results in lesions that may respond in a different manner than those studied presently.

The implementation of a 5-day pH cycle rather than a longer 20-day cycle may be another limitation. While a shorter pH cycle is less likely to completely reharden lesions, it allows for easier analysis of the different interventions post-pH cycling outcomes and for a faster secondary demineralization to occur.[12] However, a shorter cycle may inadequately represent the natural de- and remineralization processes in the oral cavity, curtailing our results. Additionally, had we altered our daily pH cycle regimen to have less time in the remineralization solution, more closely mimicking a high caries risk patient whose oral cavity is in constant demineralization, our findings may have been much different with little surface rehardening experienced, making it difficult to observe both the re- and demineralization effects of our study. While our study did not evaluate the dark staining associated with SDF, it would be interesting to investigate whether there is a correlation between the color change in specimens and the extent of surface rehardening.

Despite the limitations encountered in our study, we found SDF to be an effective intervention to reharden the surface of early, incipient, noncavitated, white-spot lesions in enamel. However, SDF was not as effective as FV in preventing surface softening in these lesions after a secondary demineralization challenge. In an effort to better understand the effects of SDF on early enamel lesions, we believe it would be beneficial for future research to incorporate cariogenic biofilm models and a wider range of FV products.

CONCLUSION

Based on our findings, FV may be better suitable than SDF to treat early, incipient, noncavitated, white-spot, enamel caries lesions. Further research is needed on SDF, and its prevention of enamel surface softening upon exposure to demineralization challenges before SDF can be recommended over FV in the treatment of early, incipient, noncavitated, white-spot, enamel caries lesions.

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Conflict of Interest

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Adhesion to a Zirconia-reinforced Lithium Silicate Ceramic: Effects of Ceramic Surface Treatments and Resin Cements

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Clinical Relevance

The bond strength of CAD/CAM zirconia-reinforced lithium silicate (ZLS) glass-ceramic is influenced by applying different ceramic surface treatments and using different types of resin cements. Such steps need to be carefully selected to achieve an optimized adhesion to the ZLS ceramic.

SUMMARY

Objective: This study had the objective to test the effect of ceramic surface treatments on the microshear bond strength (μSBS) of different resin cements to a zirconia-reinforced lithium silicate (ZLS).

Methods and Materials: ZLS blocks were sectioned, embedded in acrylic resin, and then allocated into nine groups considering two study factors: "ceramic surface treatment" (HF - hydrofluoric acid; EP - self-etching primer; TBS - tribochemical silica coating) and "resin cements" (nMDP - without MDP monomer; MDP - with MDP monomer; SA - self-adhesive). Starch tubes (n=36) were placed on the treated ceramic surface and the cement was applied. Starch tubes were removed after 24 hours of storage, and the specimens were thermocycled

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(5,000×; 5°C-55°C). Next, the μSBS test was performed using the wire-loop technique, and topographic and failure analyses were performed.

Results: The factors "ceramic surface treatment" and "resin cement" statistically influenced the μSBS results. Considering the surface treatment factor, the TBS produced statistically lower values when the MDP resin cement was applied, being only similar to the MDP plus EP group. For the resin cement factor, no difference was found for nMDP and SA groups, apart from the surface treatments. Failure analysis showed that the groups treated with EP had a greater number of pre-test failures. The surface treatments induced noteworthy topographic alterations when compared to control (no treatment).

Conclusion: The ZLS ceramic surface treatment with tribochemical silica coating associated with the MDP-containing resin cement resulted in lower bond strength values.

INTRODUCTION

Monolithic ceramic restorations have been widely used with current advances in dentistry, mainly through the development of Computer-Aided Design/Computer-Aided Machining (CAD/CAM) technology, which has enabled clinicians to provide precise high-quality ceramic restorations.[1,2] Zirconia-reinforced lithium silicate (ZLS) glass-ceramic is a good option for a variety of clinical applications, since it provides good aesthetic and mechanical performance.[3] This material is a glass-ceramic reinforced by lithium-metasilicate (Li_2SiO_3) crystals with zirconia crystals (ZrO_2; 8-14 wt%)[4] dissolved in the glassy matrix.[5,6]

Ceramics should have good bonding strength to resin cements in addition to good mechanical properties to provide long-term success of full-contour ceramic restorations. In this sense, the surface treatment of glass-ceramics plays an important role. This procedure improves the surface-free energy, increasing the surface wettability for the cements, in turn favoring the micromechanical and chemical bonding between the resin cement and glass-ceramics.[7,8]

Hydrofluoric (HF) acid etching of glass-ceramics is well established in the literature as an effective surface treatment, since it selectively dissolves the vitreous matrix, creating micropores on the surface into which the resin cement penetrates, promoting mechanical interlocking.[2,9] In addition, the exposure of the silica crystals interacts with the silane agent which is copolymerized with methacrylate groups of the resin cement's organic matrix.[10] However, according to Al-Thagafi and others,[11] tribochemical silica coating by air-abrasion silica-coated alumina particles presents superior bond strength results compared to 5% HF etching with ZLS ceramic. In this sense, tribochemical silica coating would be a good alternative for ceramic surface treatment.[12]

Another surface treatment is self-etching ceramic primers (all-in-one approach) which have emerged as a less toxic and easy-to-apply technique that enables surface etching and silanization in one single step, reducing the clinical time and technical sensitivity.[13] These primers also selectively etch the ceramic surface, however different from HF acid etching. These primers only promote small changes in the ZLS ceramic surface,[7] which may impair the interlocking of resin cement tags with the ceramic surface.[14]

In addition to an efficient surface treatment, the cement is also important for long-lasting tooth/restoration bonding.[15] In an attempt to provide easy and fast handling, as well as a less-sensitive bonding technique, distinct simplified resin cement systems (ie, self-adhesive) have emerged in addition to conventional resin cements. Self-adhesive resin cements bond to the dental substrate without the need for pretreating the tooth, since they enable chemical bonding between the self-adhesive resin and the hydroxyapatite,[16,17] but still require a ceramic surface pretreatment.[18]

On the other hand, conventional resin cements require a bonding agent between their resinous matrix and the dental surface.[19] Furthermore, some cements have bifunctional phosphate monomers (ie, 10-methacryloxydecyl dihydrogen phosphate — 10-MDP) in their composition which chemically bond to the ceramic surface's oxides through a phosphate ester group,[17] and to the resin matrix of the cement through a methacrylate group.[20] Although some studies indicate that MDP-containing resin cements show good performance in the bond strength to different ceramic materials,[21-23] according to Secilms and others,[24] a conventional resin cement without MDP presented better bond strength results than a cement with MDP. However, there is a lack of studies in the literature evaluating adhesion to ZLS ceramics exploring the effects of surface treatments and distinct resin cements to evidence the adhesion outcome when combining these two factors.

Thus, as the literature is still inconclusive about this important topic which plays a relevant clinical role for ceramic performance under clinical service, the purpose of this *in vitro* study was to evaluate the influence of ceramic surface treatments on the microshear bond strength of different resin cement systems to a zirconia-

reinforced lithium silicate glass-ceramic. The null hypotheses were: 1) the surface treatments and 2) the resin cement type would not influence the final bond strength results.

METHODS AND MATERIALS

The main information about the materials used in this study is described in Table 1.

Preparation of Ceramic Specimens

A total of 27 rectangular slices (14×12×1 mm^3) were cut from zirconia-reinforced lithium silicate glass-ceramic blocks (18×14×12 mm^3; Vita Suprinity, Vita Zahnfabrik, Bad Säckingen, Germany) with a diamond blade in a cutting machine (Isomet 1000, Buehler, Lake Bluff, Illinois, USA) under constant water cooling. The slices were manually polished on both sides with silicon carbide papers having different grit sizes (#400, #600, and #1200-grit; Norton Abrasives, Saint-Gobain; São Paulo, SP, Brazil). Next, one side (bonding surface) of each specimen was manually ground (15 times in each axis, *x* and *y*) with a #60-grit size silicon carbide sandpaper (Norton Abrasives, Saint-Gobain) by applying light finger pressure to simulate the roughness obtained from CAD/CAM milling, thus providing a mean roughness (Ra=1.98 μm and Rz=12.88 μm)

Table 1: Materials Used in the Study and Respective Characteristics (Commercial Name, Manufacturer, and Chemical Composition)

Material and Manufacturer	Composition
Zirconia-reinforced lithium silicate glass-ceramic (VITA Suprinity, VITA Zahnfabrik, Bad Säckingen, Germany)	SiO_2; Li_2O; K_2O; P_2O_5; ZrO_2; Al_2O_3; CeO_2; pigments
Hydrofluoric acid 5% (IPS Ceramic Etching-gel, Ivoclar, Schaan, Liechtenstein)	Hydrofluoric acid < 5%
Self-etching ceramic primer (Monobond Etch & Prime, Ivoclar)	Tetrabutyl ammonium dihydrogen trifluoride, methacrylated phosphoric acid ester, trimethoxysilylpropyl methacrylate, alcohol, water
30 μm silica-coated alumina particles (CoJet Sand, 3M ESPE, Seefeld, Germany)	Aluminum oxide. Free amorphous synthetic silica
MDP-free conventional resin cement (Multilink Automix, Ivoclar)	Base: ytterbium trifluoride, ethoxylated bisphenol A dimethacrylate, Bis-GMA, 2-HEMA, 2-dimethylamanoethyl methacrylate. Catalyst: ytterbium trifluoride, ethoxylated bisphenol A dimethacrylate, urethane dimethacrylate, 2-HEMA, dibenzoyl peroxide and silica filler (68 wt%),[25] pigments
MDP-containing conventional resin cement (Panavia F 2.0 - Kuraray Noritake, Ukayama, Japan)	10-methacryloxydecyl dihydrogen phosphate, bisphenol-A-polyethoxy dimethacrylate, hydrophobic aliphatic methacrylate, hydrophilic aliphatic methacrylate, silanated silica filler, silanated barium glass filler (78 wt%),[26] sodium fluoride
Self-adhesive resin cement (RelyX U200 - 3M ESPE)	Base paste: Methacrylate monomers containing phosphoric acid groups, Methacrylate monomers, silanated fillers, initiator components, stabilizers, rheological additives. Catalyst paste: Methacrylate monomers, alkaline (basic) fillers, silanated fillers (72 wt%),[27] initiator components, stabilizers, pigments, rheological additives
Silane coupling agent (Prosil - FGM, Joinville, Brazil)	3-methacryloxipropyltrimethoxysilane (<5%); ethanol (>85%); water (<10%)

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

before crystallization similar to the intaglio surface of CAD/CAM milled restorations.[28] The specimens were washed in an ultrasonic bath (1440 D, 50/60 Hz, Odontobras, Ind And Com Equip Med Odonto LTDA, Ribeirão Preto, Brazil) with distilled water for 10 minutes, air-dried for 30 seconds, and crystallized in a specific furnace according to the manufacturer's instructions (VACUMAT 6000 MP, Vita Zahnfabrik, 840°C, 8 min vacuum).

Each ceramic slice was embedded in a cylindrical polyvinyl chloride (PVC) mold using a self-cured acrylic resin (VIPI Flash, Pirassununga, Brazil). For this, a double-sided tape (3M Sumare, Brazil) was used to keep the bonding surface free for cementation. After the final polymerization of the acrylic resin, the specimens were washed in an ultrasonic bath (as previously reported) to remove any glue residue. The slices were randomly assigned (www.randomizer.org) into nine groups (three ceramic slices per group) according to the study factors: surface treatment and type of resin cement, as shown in Table 2.

Ceramic Surface Treatments

Hydrofluoric Acid Etching (HF)—Hydrofluoric acid (5% IPS Ceramic Etching Gel, Ivoclar, Schaan, Liechtenstein) was applied and scrubbed on the ceramic surface with a microbrush for 20 seconds. As recommended by the manufacturer, the specimens were washed with air/water-spray for 30 seconds, then subjected to an ultrasonic bath (1440D, Odontobras) with distilled water for 5 minutes, and air-spray dried for 30 seconds. A silane coupling agent (Prosil, FGM, Joinville, Brazil) was actively applied with a microbrush for 15 seconds, kept to react for 60 seconds, and gently air-spray dried for 15 seconds.

Etch and Prime Ceramic Primer (EP)—The self-etching ceramic primer (Monobond Etch & Prime, Ivoclar) was actively applied on the ceramic surface with a microbrush for 20 seconds, kept to react for 40 seconds, washed with air/water-spray for 20 seconds, and air-spray dried for 30 seconds.

Tribochemical Silica Coating (TBS)—The tribochemical silica coating was performed with 30 μm silica-coated aluminum oxide particles (CoJet Sand, 3M ESPE, Seefeld, Germany) using a micro-etcher (DENTO-PREP microblaster, Ronvig, Daugaard, Denmark) at a distance of 15 mm from the device's nozzle to the ceramic surface with a pressure of 2.5 bar[29] in oscillatory movements for 15 seconds.[11] A gentle air-spray was subsequently applied to remove the loose particles, and a silane coupling agent (Prosil, FGM) was actively applied for 15 seconds, kept to react for 60 seconds, and gently air-spray dried for 15 seconds.

Table 2: *Study Design and Study Groups*

Surface Treatments	Resin Cements	Group Codes
5% hydrofluoric acid etching (IPS Ceramic etching-gel) + Silane (Prosil) - HF	Multilink Automix - nMDP	nMDP+HF
	Panavia F 2.0 - MDP	MDP+HF
	RelyX U200 -SA	SA+HF
Self-etching ceramic primer (Monobond Etch & Prime) - EP	Multilink Automix - nMDP	nMDP+EP
	Panavia F 2.0 - MDP	MDP+EP
	RelyX U200 -SA	SA+EP
Tribochemical silica coating (CoJet Sand) + Silane (Prosil) - TBS	Multilink Automix - nMDP	nMDP+TBS
	Panavia F 2.0 - MDP	MDP+TBS
	RelyX U200 -SA	SA+TBS

Abbreviations: nMDP, MDP-free conventional resin cement; MDP, MDP containing conventional resin cement; SA, self-adhesive resin cement; HF, 5% hydrofluoridric acid; EP, self-etching ceramic primer; TBS, tribochemical silica coating

Microshear Resin Cement Sample

After the ceramic surface treatment, starch tubes (Renata, Pastificio Selmi, Londrina, Brazil) with 1.0 mm of height and 0.96 mm of internal diameter were placed on the treated ceramic surface and fixed at their external surface with sticky wax (Lysanda, São Paulo, Brazil) to keep them in position (n=36; 12 starch tubes in each ceramic slice, with 3 ceramic slices for each group).[30]

The adhesive procedures were performed at room temperature (25°C) by a single trained operator, and the resin cements were manipulated and applied according to the manufacturer's recommendations, as follows.

MDP-Free Conventional Resin Cement (nMDP- Multilink Automix)—The resin cement base and catalyst pastes were dispensed from the double-push syringe, then mixed for 20 seconds resulting in a homogeneous mixture, and inserted with a probe into the starch tubes.

MDP-Containing Conventional Resin Cement (MDP-Panavia F2.0)—The resin cement base and catalyst pastes were dispensed from the syringes, then mixed for 20 seconds resulting in a homogeneous mixture, and inserted with a probe into the starch tubes.

Self-Adhesive Resin Cement (SA - RelyX U200)—The resin cement base and catalyst pastes were dispensed from the double-push syringe, then mixed for 20

seconds resulting in a homogeneous mixture, and inserted with a probe into the starch tubes.

The resin cement excesses were carefully removed with a microbrush. The cement of each starch tube was then light-activated (1200 mW/cm^2 of intensity, Radii Cal, SDI, Bayswater, Australia) for 40 seconds, and the specimens were stored in distilled water in a laboratory incubator at 37°C for 24 hours. The starch tubes were then carefully removed with a clinical probe. The adhesive interface of all the samples was analyzed in a stereomicroscope (Stereo Discovery V20, Carl-Zeiss, Gottingen, Germany) to inspect the integrity of the adhesive zone, and the samples presenting any bubbles or defects were discarded and replaced.

Aging—Thermocycling

After storage for 24 hours, all specimens underwent intermittent 5000 thermal-cycles (Ethik Technology Limited — model 521-6D; Vargem Grande Paulista, SP, Brazil) with the temperature ranging from 5°C to 55°C with 30 seconds of dwell time at each temperature and 4 seconds of transfer time.[3]

Microshear Bond Strength Test—Wire-Loop Method

The PVC cylinders were placed in a jig attached to a universal testing machine (Emic DL1000, São José dos Pinhais, Brazil) so that the cement cylinder was in alignment with the center of the load cell, being parallel to the adhesive interface. A stainless-steel wire (Ø=0.20 mm) was looped around the specimen cylinder parallel to and as close as possible to the cement-ceramic interface. The shear load was applied (10 N load cell) at a rate of 0.5 mm/min until failure occurred, and the data were recorded in MPa.

Failure Analysis

All tested specimens were inspected in a stereomicroscope (Discovery V20, Carl-Zeiss, Gottingen, Germany) with 10-50× magnification to verify the failure type. The failures were classified as adhesive (less than 50% of resin cement remained at the adhesive interface) or cohesive (more than 50% of resin cement remained in the adhesive interface). Representative samples were selected and analyzed in a scanning electron microscope (SEM) (Secondary electrons, 20kV; VEGA3, Tescan, Brno, Czech Republic) at 100× and 230× magnification.

Topographic Analysis

Two additional specimens of each group were produced, treated as mentioned above, dried in a laboratory desiccator, and gold-sputtered to be analyzed in an SEM (Secondary electrons, 20kV; VEGA3, Tescan) to evaluate the ceramic surface characteristics after the distinct treatments.

Data Analysis

According to normality (Shapiro-Wilk) and homoscedasticity (Levene's) tests, the data were nonparametric and heterogeneous. Thus, the bond strength was analyzed using the Kruskal-Wallis nonparametric test and Dunn's post-hoc test at a significance level of 0.05. The samples having cohesive or pre-test failures were disregarded from the study and the data analysis. Each cement cylinder was defined as an experimental unit, and the sample size was 36 for each testing group (n=36).

RESULTS

According to the Kruskal-Wallis ($p<0.001$) and Dunn's tests (standard error for comparison: 16.648 to 22.942; critical Z value: 3.197; critical value for comparison: 53.223 to 73.346), the microshear bond strength results were statistically influenced by the ceramic surface treatment and by the resin cement factors. The tests showed that there was no statistical difference between nMDP and SA resin cements, regardless of the surface treatment used. However, for the conventional resin cement containing MDP, the surface treatment with HF etching promoted the highest bond strength values (mean: 7.22; median: 6.13 MPa), being statistically similar to EP (mean: 5.27; median: 5.78 Mpa), that was similar to TBS (mean: 2.63; median: 1.77 Mpa). Comparing the resin cements when applying the same ceramic surface treatment, there was only one difference when the MDP cement was used with TBS, which presented statistically lower values than the other groups (mean: 2.63; median: 1.77 MPa) (Table 3)— although it presented the highest standard deviation proportionally in relation to the mean of the study.

Failure at the adhesive zone ("adhesive") was the main type of failure of the samples. (Figures 1 and 2). However, the groups treated with the self-etching ceramic primer showed a considerably higher number of pre-test failures due to debonding (failure at the ceramic-resin interface) (Figure 1), especially the group MDP+EP. The group that presented the highest number of cohesive failures was the SA+HF, having no pre-test failure.

In terms of topographic changes, tribochemical silica coating created a more irregular surface, introducing sharper flaws and scratches. The hydrofluoric acid etching promotes alterations at the nanoscale by removing the nanometric grains, while the self-etching

Table 3: *Mean, Standard Deviation (SD), and Median (First and Third Quartiles - Q1-Q3) Values of Microshear Bond Strength in MPa (Kruskal-Wallis and Dunn's Post-Hoc Tests)*

Resin Cements	Means and Medians	Surface Treatments[a]		
		HF	EP	TBS
nMDP	Mean (SD)	6.48 (3.86) Aa	5.07 (2.48) Aa	8.54 (4.01) Aa
	Median (Q1-Q3)	4.97 (4.4- .81)	4.71(3.65-6.62)	8,76 (5.99-11.33)
MDP	Mean (SD)	7.22 (4.04) Aa	5.27 (4.21) ABa	2.63 (2.62) Bb
	Median (Q1-Q3)	6.13 (4.4-9.25)	5.78 (0.53-9.25)	1.77 (0.74-3.6)
SA	Mean (SD)	11.93 (6.8) Aa	10.1 (7.11) Aa	7.1 (3.85) Aa
	Median (Q1-Q3)	9.1 (7.12-19.27)	9.21 (2.96-16.49)	6.53 (3.69-10.01)

Abbreviations: nMDP, MDP-free conventional resin cement; MDP, MDP containing conventional resin cement; SA, self-adhesive resin cement; HF, 5% hydrofluoridric acid; EP, self-etching ceramic primer; TBS, tribochemical silica coating.

[a]Different uppercase letters show statistically significant differences between surface treatments for the same resin cement (p<0.05) (lines). Different lowercase letters show statistically significant differences between the resin cements for the same surface treatment (p<0.05) (columns).

ceramic primer (mild acid) induces lower topography alterations (Figure 3).

DISCUSSION

The null hypotheses were rejected once the bond strength to a ZLS ceramic was directly influenced by the surface treatment and the type of resin cement applied. The worst scenario in terms of adhesive strength of ZLS ceramics occurred when treating with the silica coating tribochemical method and luting with the resin cement containing MDP concomitantly.

The groups treated with Monobond Etch & Prime—which is composed of a silane, a ceramic agent, and a priming agent (Table 1) in a single bottle—produced statistically similar results to the HF etching, regardless of the cement used, corroborating the results presented in the literature.[13,31,32,33] However, all groups that received this surface treatment had the highest number of pre-test failures (Table 3; Figure 1). This can be corroborated by the topography images of the ceramic that show a different result between both treatments (EP and HF). This demonstrates that the initial characteristics (CAD/CAM milling simulation) remain after EP surface treatment, while HF etching promoted dissolution of the surface glassy matrix, thereby removing the defects created by the milling simulation and more uniformly exposing the ceramic glassy matrix, making the surface more reactive and prone to adhesion with the resin cements (Figure 3). Prado and others[34] also observed a greater number of pre-test failures for a lithium disilicate and a feldspathic ceramic treated with such ceramic primer, but they have also observed that the Monobond Etch & Prime self-etching ceramic primer produced stable bonding after aging.

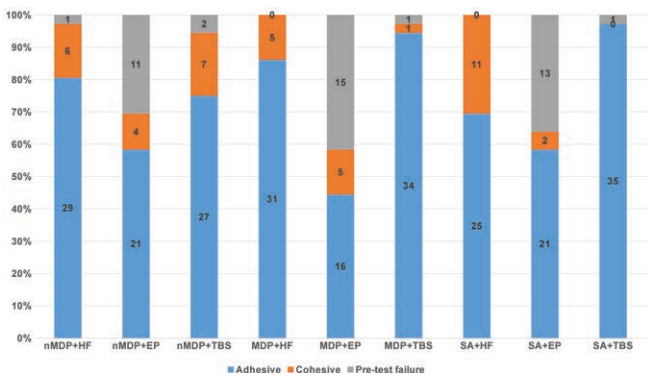

Figure 1. *Number and percentage for types of failure and pre-test failures of each experimental group submitted to the microshear bond strength test.*

Tribochemical silica coating treatment showed similar bond strength values to HF for the MDP-free conventional and self-adhesive resin cements. Al-Thagafi and others[11] also found an improvement in adhesive strength using the tribochemical silica coating (CoJet Sand, 3M ESPE) followed by silanization of the ZLS ceramic, however using this protocol for the repair of the ceramic with composite resin. Yet, Sato and others[29] showed a significant decrease in the bond strength after thermocycling when the zirconia-reinforced lithium silicate ceramic was treated with tribochemical silica coating (CoJet Sand), suggesting that this surface treatment does not provide a stable bond. Altan and others[35] also concluded that the surface treatment with tribochemical silica coating is not as effective as the HF etching in terms of bond strength to the ZLS ceramic. In addition, a rougher

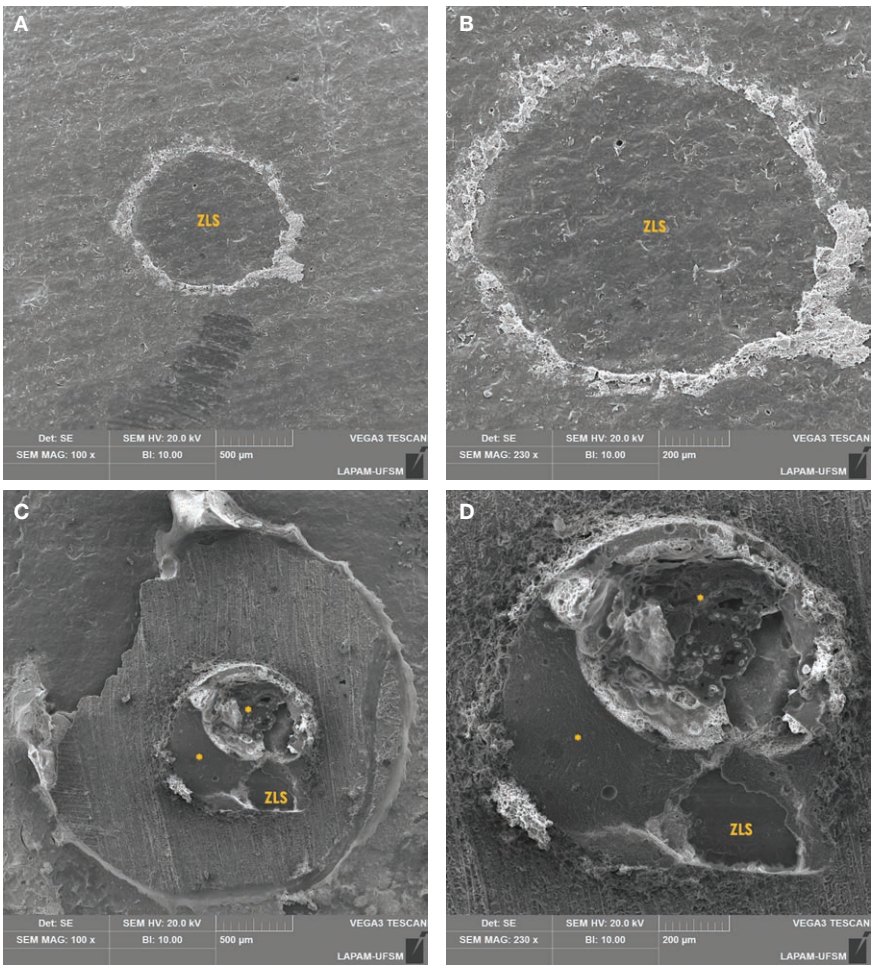

Figure 2. *SEM images (100× and 230× of magnification) of the different types of failure after the microshear bond strength test: adhesive (A-B) and cohesive (C-D). ZLS (zirconia-reinforced lithium silicate ceramic); '*' remnant of the resin cement.*

ceramic surface, such as that presented by the air-abraded groups (Figure 3), can have a deleterious effect on mechanical properties when defects are not properly filled by the adhesive bonding.[36-38]

The bonding between two materials does not depend only on the size of the irregularities created for the surface treatment, but also on the ability of cements to infiltrate them.[10] No statistical difference was found between MDP-free and self-adhesive resin cements used in the study, regardless of the surface treatment applied (Table 3). The lowest value of bond strength was observed when the MDP-containing conventional resin cement was used in association with the TBS surface treatment, being statistically similar to EP (Table 3) for the same cement. Zeller and others[39] found that a conventional resin cement with MDP has a higher viscosity at the beginning of its manipulation at room temperature (23°C), which decreases during the temperature increasing (37°C = mean oral temperature), simulating the manipulation and clinical adjustments during cementation of a restoration. However, in *in vitro* studies, this increase in temperature does not happen, maintaining the increased viscosity of the resin cement during its handling, which can hinder its penetration into the roughness created by the air-abrasion (Figure 3).

The majority of failures found between the groups were adhesive, however, the EP groups (nMDP+EP, MDP+EP, SA+EP) showed the highest number of pre-test failures, which is considered the main limitation of this study. This may be explained due to the lower chemical interaction between this surface treatment and the resin cements, which may preclude a durable micromechanical bonding.[34,40] These failures were disregarded from statistical analysis, which may have overestimated the bond strength values of the EP groups since no value was given to the pre-test failures[41,42] because they increased the standard deviation. Therefore, the analysis of the data of the EP groups should be used with caution.

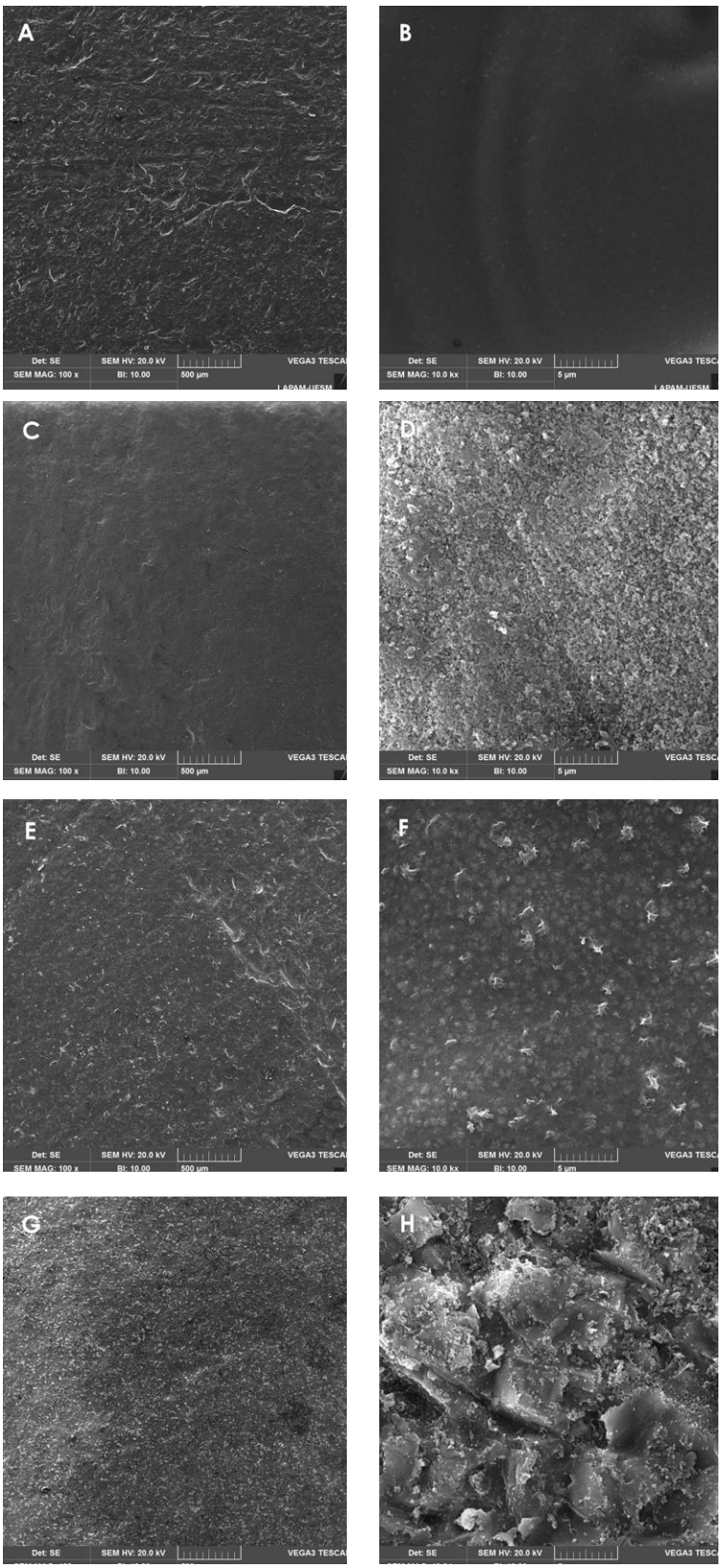

Figure 3. *Topographic images on scanning electron microscopy (100× and 10,000× of magnification) of the ceramic surface on control (CAD/CAD milling simulation, without surface treatment) and the surface treatments: Control (A-B); 5% hydrofluoric acid etching (HF) (C-D); self-etching ceramic primer (EP) (E-F); and tribochemical silica coating (TBS) (G-H).*

Another limitation was the number of cohesive failures. According to Braga and others,[41] such failures are explained by the test mechanics and fragility of the materials involved. Della Bona and van North[43] showed that the microshear bond strength test creates a non-homogeneous load distribution at the interface, which is associated with distal failures in the adhesive interface. Furthermore, cohesive failures often mean that the bond within the material itself is weaker than the bond at the interface,[24] justifying the high microshear bond values in such groups. Thus, in order not to overestimate the bond strength to the material, the values of cohesive failures were not included in the statistical analysis.

Future *in vitro* studies should be conducted to assess other bonding approaches by exploring distinct resin cements, adhesive agents, and glass-ceramics.

CONCLUSIONS

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

- The bond strength to the zirconia-reinforced lithium silicate glass-ceramic is significantly influenced by the ceramic surface treatment performed and the resin cement used.
- The surface treatments with hydrofluoric acid and ceramic primer were similar to each other regardless of the resin cement used.
- The performance of resin cement without MDP and self-adhesive were similar to each other, regardless of the surface treatment used.
- Using the MDP-containing resin cement after treating the ceramic surface with the tribochemical silica coating method resulted in lower bond strength values among the tested conditions.

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Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article

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Color Changes Associated with Sandblasting, Hydrofluoric Acid Etching, and Er:YAG Laser Irradiation of Milled Feldspathic Porcelain Laminate Veneers

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Clinical Relevance

The Er:YAG laser irradiation can be considered as a biologically and environmentally safe method for surface treatment of thin, milled feldspathic porcelain veneers.

SUMMARY

Objectives: To evaluate color changes in milled feldspathic porcelain laminate veneers following hydrofluoric acid etching (HFA), sandblasting (SB), or Er:YAG laser irradiation (LI).

Methods and Materials: Disc-shaped specimens (thickness=1 mm, diameter=8 mm) were milled from feldspathic porcelain blocks (n=40). Glazed specimens were randomly assigned to four subgroups (n=10 each) according to surface treatment: negative control, HFA, SB, and LI. A layer of translucent, light-cured resin cement (thickness=0.1 mm) was then applied following silanization. The color was characterized by the L*, a*, and b* uniform color space (CIE) using a reflection spectrophotometer. CIEDE2000 (ΔE_{00}) was calculated to determine the color difference between each surface treatment and negative control groups. Data were statistically analyzed using analysis of variance (ANOVA), Kruskal-Wallis, and Dunn-Bonferroni post hoc tests.

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Results: There were no significant differences in CIEL* and CIEb* coordinates between negative control and all surface treatment groups ($p \geq 0.108$). The SB group demonstrated significantly lower mean CIEa* (higher greenish hue) compared to other groups ($p \leq 0.003$). HFA exhibited significantly higher CIEa* (closer to red) when compared to LI ($p=0.039$). LI induced the smallest overall color change compared to negative control ($\Delta E_{00}=1.43$ [1.07]). However, the differences in ΔE_{00} values were not statistically significant ($p=0.648$).

Conclusions: The tested surface treatments did not affect the lightness or the yellowness of the 1-mm-thick milled feldspathic porcelain veneers. However, sandblasting resulted in a significant increase in the greenish hue. The Er:YAG laser resulted in the closest ΔE_{00} (1.43) to the 50:50% perceptibility threshold ($\Delta E_{00}=1.2$).

INTRODUCTION

Laminate veneers (LVs) have gained wide popularity as a minimally invasive alternative to full coverage crowns. They can be effectively utilized to treat aesthetic disharmonies, including discrepancies in tooth size, position, shape, contour, alignment, and bleaching-resistant tooth discoloration.[1] Further, LVs have functional indications as they can be used for improving phonetics and incisal guidance.[1] LVs require no or minimal tooth preparation and can be adhesively bonded to the tooth structure and thereby can be effectively used in tooth surface loss cases.[2,3] Long-term clinical data demonstrated remarkably high success/survival rates, thanks to the ever-improving ceramic materials and adhesive bonding systems.[1,4]

Feldspathic porcelains have long been utilized to produce lifelike and highly aesthetic LVs due to their optimum optical properties.[1] The superior mechanical properties of leucite and lithium disilicate glass-ceramics expanded the applications of LVs.[5,6] Clinical studies demonstrated that reinforced glass-ceramic LVs are associated with higher survival/success rates compared to feldspathic porcelain counterparts.[7] The introduction of translucent zirconia allowed the production of ultrathin and yet mechanically reliable LVs.[8,9] Indirect resin-based composites attracted attention as a potential substrate for the construction of LVs for being less brittle and easier to repair when compared to ceramics.[10-12]

The widespread application of computer-assisted design/computer-assisted manufacturing (CAD/CAM) has revolutionized the fabrication of LVs. CAD/CAM technology can be utilized to fabricate full-contour monolithic LVs, cut-back cores ready to receive veneering porcelain, or wax patterns to be used for the production of any of the aforementioned using a heat-pressing technique. CAD/CAM allowed single-visit, chair-side fabrication of LVs for patients with pressing social and professional commitments and eliminated interappointment microleakage.

Adhesive bonding of LVs with resin composite cements to tooth structure is a key determinant for success and longevity.[7] Successful bonding requires appropriate surface modification of the fitting ceramic surface and conditioning of the tooth structure. Roughening of the fitting surface of LVs promotes micromechanical retention via the interdigitation between the ceramic material and the resin composite cement. Further, surface modification may promote chemical adhesion by increasing reactivity to the silanization process via condensation of the silanol groups in the vicinity of hydroxylated silica groups.[13] Glass-containing ceramics can be effectively treated chemically or mechanically. The amorphous glassy phase is highly susceptible to chemical dissolution by hydrofluoric (HF) acid.[14,15] Sandblasting can also effectively create the required microroughness.[16] HF acid is considered a biologically and environmentally hazardous agent and may generate a significant amount of crystalline debris that contaminates the etched surface.[17] Further, the high refractive index of residual sandblasting material may alter the optical properties of the ceramic material. Hence, meticulous post-treatment cleaning should be warranted following HF acid etching or sandblasting.[17] More importantly, both techniques can be deleterious to the mechanical reliability of the ceramic materials.[18-20]

Lasers have been considered as an effective and safe method for ceramic surface treatment.[21-24] The high energy delivered by laser irradiation leads to thermally or chemically induced morphological surface alterations. Several types of lasers have been advocated for etching dental ceramics, including CO_2, Nd:YAG, and Erbium (Er) lasers.[25] Several studies reported adequate resin bond strength to ceramic substrates following laser irradiation, while others demonstrated suboptimal performance.[25] The disparity in the findings can be attributed to the variations in the implemented techniques and irradiation parameters.

The Er:YAG laser is a solid-state laser that emits light within the infrared spectrum (2940 nm). It is widely used in dentistry as it is highly absorbed by hydroxyapatite.[25] Several studies have investigated the use of Er:YAG irradiation as a surface treatment to enhance adhesive resin bonding. Er:YAG laser irradiation resulted in inferior resin bond strength

to feldspathic porcelain compared to HF/silane.[26-29] Aksakalli and others however, reported comparable resin bond strength to feldspathic porcelain upon applying HF/silane or Er:YAG irradiation surface treatments.[30] Higher, but not significantly different, resin shear bond strength to feldspathic porcelain was reported with Er:YAG laser irradiation compared to HF/silane.[31] The effect of an Er:YAG laser on surface roughness of, and resin bond strength to, leucite and lithium disilicate glass-ceramics and zirconia appears to be more pronounced.[22, 24,32-34]

The overall color of ceramic reconstruction can be influenced by: 1) reconstruction thickness,[35,36] 2) shade and film thickness of the luting agent,[35,36] and 3) color of the underlying tooth structure.[37] Polishing/glazing of the restoration's cameo surface may also affect the color, translucency, and texture of ceramic reconstruction.[38] Treatment of the fitting surface may also alter optical properties due to chemical/morphological alterations that may affect the refractive index or thickness of the reconstruction. One study reported a significant reduction of translucency of thin (thickness=0.5 mm), pressed lithium disilicate glass-ceramic LVs upon using Er:YAG laser or sandblasting compared with HF acid etching.[39]

Currently, there is limited evidence pertaining to the color changes with various surface treatments of contemporary CAD/CAM feldspathic porcelain substrates. The objective of this study was to compare color changes associated with the Er:YAG laser, sandblasting, and hydrofluoric (HF) acid surface treatments when used on CAD/CAM porcelain LVs. The null hypothesis of this study was that all surface treatments will not induce a significant color change in the tested feldspathic porcelain substrate.

METHODS AND MATERIALS

A commercially available CAD/CAM feldspathic ceramic substrate (VITABLOCS Mark II, VITA Zahnfabrik, Bad Säckingen, Germany) was used in this study as the LV material (shade A1). Forty disc-shaped specimens (diameter=8 mm, thickness=1 mm) were digitally designed (Ceramill Mind, Amann Girrbach AG, Koblach, Austria) and milled from fully sintered blocks using a 5-axis milling unit (Ceramill Motion 2, Amann Girrbach AG). All discs were finished and glazed according to the manufacturer's instructions (VITA AKZENT Plus, VITA Zahnfabrik).

Specimens were randomly divided into four groups according to the surface treatment type (n=10 per group):

- Negative control: No surface treatment.
- HF acid etching (HFA, positive control): The bonding surface of each disc was etched with 9.5% HF acid for 60 seconds. The acid gel was rinsed with water for 20 seconds and then dried using an oil-free airstream.
- Sandblasting (SB): The bonding surface of each disc was sandblasted with 50 μm Al_2O_3 particles for 20 seconds (pressure=2.5 bar, distance=10 mm).
- Laser irradiation (LI): An Er:YAG laser (Pluser, Doctor-Smile, Lambda SpA, Brendola, Italy) was used for laser irradiation. The optical fiber was aligned perpendicularly to the bonding surface of each disc at a distance of 1 mm. The whole surface of the disc received the laser irradiation according to the following parameters: pulse energy = 400 mJ, frequency = 20 Hz, power = 10 W, energy density = 40 J/cm^2 and pulse length = 150 μs.

Following surface treatment, all discs were cleaned in a distilled water ultrasonic bath for 10 minutes. Two coats of pre-hydrolyzed silane primer (Porcelain Primer, BISCO, Inc, Schaumburg, IL, USA) were applied to each disc and then dried with an oil-free airstream. A layer of light-cured, translucent resin cement was applied to the bonding surface of each disc (Choice 2, BISCO, Inc). The cement layer was adapted using a clean glass slab, which was then placed over the cement layer under a 1-kg weight for 30 seconds. Upon removing the weight and glass slab, the cement was cured using a light-curing tip applied from the glazed ceramic side for 60 seconds. Then, the cement layer was adjusted with wet silicon carbide paper (600 grit) to 0.1 ± 0.01 mm, yielding 1.1-mm-thick discs in all groups. An electronic digital caliper was used to verify thickness at various stages of the specimen preparation process (JOCAL, CE Johansson AB, Eskilstuna, Sweden).

A reflection spectrophotometer device was used for color measurements (VITA Easyshade, VITA Zahnfabrik) with white background and under a D65 (daylight) illuminant while specimens were placed in a lightbox to standardize the external lighting conditions. The spectrophotometer's probe was placed in the center of each disc, and measurement was repeated three times for each disc. For each specimen, a mean value was calculated for the three color coordinates of the Commission Internationale de l'Eclairage (CIE) system; L*: lightness: black-white; a*; greenish-redness; and b*: blueness-yellowness. Mean chroma or color saturation index (C) and hue angles were also calculated for all groups. The perceptible color difference metric CIEDE2000 (ΔE_{00}) was calculated using the following equation:

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

The Shapiro-Wilk test indicated that some data sets did not follow a normal distribution ($p<0.05$). One-way analysis of variance (ANOVA) and Kruskal-Wallis tests were performed to examine statistically significant interactions between various surface treatments and control groups. Pairwise comparisons were performed using the Dunn-Bonferroni post hoc test ($\alpha=0.05$). All analyses were performed using the Statistical Package for Social Sciences software (Version 23, IBM SPSS Statistics, Armonk, NY, USA).

RESULTS

There were no significant differences in CIE L* and CIE b* coordinates between negative control and all surface treatment groups ($p\geq0.108$). HFA and LI groups exhibited no statistically significant difference in CIE a* compared to the negative control group ($p\geq0.246$). SB resulted in a statistically significant reduction in the mean CIE a* indicating a notable increase of the greenish hue (-1.23 [0.04], $p\leq0.003$) compared to the other surface treatment and negative control groups. The LI group exhibited significantly lower CIE a* (increased greenish hue) when compared to HFA ($p=0.039$). No statistically significant differences were observed in the chroma index (C) or hue angles when comparing all surface treatment and negative control groups ($p\geq0.408$). LI induced the smallest ΔE_{00} (1.43, [1.07]), the differences in ΔE_{00} between all surface treatment groups and negative control were however, not statistically significant (p=0.648). Table 1 summarizes mean and standard deviation values of CIE L*a*b* coordinates, ΔE_{00}, chroma index, and hue angle for all surface treatment and negative control groups.

DISCUSSION

The purpose of this laboratory study was to investigate the color changes associated with three surface treatment methods used on milled feldspathic porcelain LVs. Untreated samples were used as a negative control to compare the effect of HFA etching, sandblasting, and Er:YAG laser surface treatments on CIE L*a*b* color coordinates. Specimens were digitally designed to standardize the thickness (1 mm). Optimum color, translucency, and texture can be achieved with 0.5-1 mm thickness LVs.[40] Further, the manufacturer recommends 0.7-mm minimum thickness of the material used in this study at the incisal third of the LV. Thus, we investigated 1-mm-thick specimens to assess color changes that can be encountered in a clinical setting. A single experienced operator performed all surface treatments and color measurements to ensure standardized procedures. The used parameters for the three surface treatments in this study were reportedly associated with the highest resin bond strength or least reduction of mechanical reliability.[13,16,19,20] For laser surface treatment, the highest pulse energy (400 mJ) and the smallest possible distance between the optic fiber and specimen (1 mm) were used, as they were reportedly associated with the highest surface roughness and/or resin bond strength.[25,28,32] A digital spectrophotometer was also used in this study for objective evaluation of color changes. Such a device may be a reliable alternative or adjunct to conventional shade guides. A digital spectrophotometer allows exportation of numerical values for various CIE color coordinates, allowing precise and highly sensitive assessment of subtle color changes.[41] However, the output of such a device may vary due to inconsistent positioning of the probe tip.[42] In this study, this problem was avoided by positioning the probe tip at the center of each specimen guided by ruler measurements. ΔE_{00} was used to assess color change as it was found to correspond better to the way human observers perceive small color differences.[43]

Surface treatments, in general, may affect the color of the ceramic substrate as a result of the physical,

Table 1: *Mean (SD) Values for CIE L*a*b* Coordinates, Hue Angle, and ΔE_{00} for Surface Treatment and Negative Control Groups*[a]

	HFA	SB	LI	Control
L*	64.46 (1.31) a	65.39 (1.46) a	64.67 (1.62) a	64.67 (1.27) a
a*	-0.81 (0.14) a	-1.23 (0.05) b	-0.93 (0.24) c	-0.91 (0.06) ac
b*	5.48 (0.60) a	4.81 (0.85) a	5.04 (0.36) a	4.78 (0.85) a
C	5.37 (0.58) a	4.99 (0.80) a	5.13 (0.39) a	4.88 (0.84) a
Hue angle	179.55° (7.24)	180.65° (5.60)	180.77° (1.37)	184.74° (10.56)
ΔE_{00}/Control	1.72 (1.03) a	1.80 (0.87) a	1.43 (1.07) a	—

Abbreviations: a (green-red); b*(blue-yellow); C, chroma index; HFA, hydrofluoric acid; L* (lightness: black-white).*

[a]*Similar letters indicate lack of statistically significant differences within the same row (p>0.05).*

chemical, or mechanical interactions that lead to surface morphological alterations. The high energy delivered to the ceramic material surface upon laser irradiation can cause melting, phase transformation, micro-explosions, or bubble inclusion within the amorphous glassy phase that may alter the optical properties of the material.[25] Laser irradiation may also result in chemical reactions that can further change the surface characteristics of certain ceramic materials.[25] The null hypothesis of this study was partially rejected as sandblasting resulted in a significant increase of greenish hue of the studied porcelain LV material. The notable color changes associated with sandblasting can be attributed to the changes in the refractive index induced by such a process. Sandblasting may increase the refractive index by increasing surface roughness, altering the thickness of the material, and changing the chemical composition of the surface due to the incorporated alumina particles within the surface of the abraded ceramic substrate. From a clinical standpoint, such color change may result in a significant color mismatch, especially when using highly chromatic resin composite cements to mask discolored abutments. Contradictory findings were reported by another study, where sandblasting and Er:YAG laser irradiation resulted in significant changes of CIE L* and b* coordinates but not a* when compared to HF acid etching and control.[44] The disparity in the findings can be attributed to the differences in the chemical composition of the studied ceramic materials (leucite, lithium disilicate, and nano-fluorapatite) and the higher pulse energy (500 mJ) used in the latter study.

Overall color change (ΔE_{00}), in comparison to the negative control, was the highest with sandblasting (1.80 [0.87]) followed by HF acid etching (1.72 [1.03]). Both surface treatments resulted in clinically acceptable color changes according to the 50:50% acceptability threshold value (ΔE=2.7) determined in the ISO standards (ISO/TR 28642:2016). A nearly perfect color match in dentistry is a color difference at or below the 50:50% perceptibility threshold, which is designated as ΔE=1.2 in the same ISO standard. Er:YAG laser induced the slightest color change (ΔE_{00}=1.43 [1.07]), which was closest to the 50:50% perceptibility threshold for the perfect color match compared to the other experimental groups.

HFA is a highly toxic and corrosive agent. The inflected tissue damage is caused by three mechanisms:[45] 1) corrosive burn from H^+ ions, 2) chemical burn from F^- ions, and 3) insoluble fluoride salt formation with calcium and magnesium within the tissues. The severity of inflected damage depends on the concentration and duration of exposure.[45] Vital tissues exposed to such acid may exhibit immediate or delayed signs of tissue destruction.[45] Direct contact, inhalation, or ingestion of HFA may result in ocular, skin, gastrointestinal, pulmonary, and hard tissue damage.[45] Hence, HFA requires strictly controlled handling, storage, exposure protection, and disposal measures. Given the hazardous nature of HFA, an alternative surface treatment must be sought in order to promote a safe and environmentally friendly practice.

The Er:YAG laser is increasingly popular in various disciplines of dentistry. It is extensively used in soft and hard tissue surgical procedures, endodontics, and prosthodontics. It can be a promising alternative to HFA etching given the current study's findings and the growing evidence regarding its positive effects on surface roughness and resin bond strength of various types of ceramic substrates. The present study investigated a single CAD/CAM substrate that may limit the generalizability of the findings, which begs further research of different materials produced by other manufacturers. Further, more studies are still required to verify the effects of Er:YAG laser surface treatment on clinically related parameters, including biomechanical reliability, fatigue resistance, discoloration, and marginal and internal fit.

CONCLUSIONS

Within the limitations of this laboratory investigation, the following can be concluded:

1. No surface treatment affected the lightness or the yellowness of the 1-mm- thick milled feldspathic porcelain veneers,
2. Sandblasting resulted in a significant increase in the greenish hue. The greenish hue was less pronounced when using HF acid compared to Er:YAG laser,
3. The overall color changes induced by all investigated surface treatments compared to negative control were not significantly different. However, from a clinical standpoint, Er:YAG laser irradiation resulted in the least perceptible color change closest to the 50:50% perceptibility threshold of a perfect color match.

Conflict of Interest

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

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Impact of Immediate and Delayed Photo-activation of Self-adhesive Resin Cements on Bonding Efficacy and Water Uptake Under Simulated Pulpal Pressure

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Clinical Relevance

A 30-second delay from placement to photo-activation is sufficient to enhance the bonding of self-adhesive cements' functional monomers to dentin while overcoming the deteriorating effect of pulpal fluid flow associated with prolonged delayed photo-activation.

SUMMARY

This study investigated the effect of immediate versus delayed photo-activation on the bonding performance and water uptake of self-adhesive (SA) resin cements under simulated pulpal pressure (SPP). The occlusal dentin surface was exposed

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in 66 extracted third molars. Resin composite cylinders were cemented to dentin under SPP, with either RelyX Unicem 2 (RU) (3M Oral Care, St Paul, MN, USA) or Maxcem Elite (MC) (Kerr, Orange, CA, USA). Each cement group was equally divided into three groups (n=8 each) according to the time elapsed between placement and photo-activation: immediate activation (IM), 30-second delayed activation (D30), or 120-second delayed activation (D120). Shear bond strength (SBS) was measured, and the type of failure was determined using a stereomicroscope. Three additional samples from each experimental subgroup were used for confocal laser scanning microscopy (CLSM) analysis. A fluorescent dye solution was added to the pulpal fluid reservoir, then a CLSM was used to detect the dye distribution within the tooth-restoration interface. Two-way analysis of variance (ANOVA) and the Tukey post-hoc test were used to analyze the SBS results (α=0.05). D30 resulted in a significantly higher mean SBS in the two cement groups than IM and D120 (p<0.05). RU showed significantly higher SBS values than MC regardless of the time of light activation (p<0.05). RU showed less dye uptake confined to the cement-dentin interface compared to the MC groups, which showed dye uptake throughout the entire thickness of the cement layer and gap formation at the interface, especially in the D120 group. The 30-second photo-activation delay group significantly improved the bond strength of SA cements. Delaying the photo-activation to 120 seconds increased pulpal fluid uptake by SA cements and compromised the integrity of the bonded interfaces.

INTRODUCTION

The clinical performance of contemporary adhesive indirect restorations depends mainly on the successful bonding of adhesive cements to tooth substrates and restorations.[1–4] Nevertheless, given the diversity of their uses in restorative dentistry and the comparatively simple handling technique, many practitioners prefer to use self-adhesive (SA) resin cements over conventional adhesive resin cements. This SA generation does not require any of the steps associated with conventional resin cements, such as acid-etching and the application of adhesive resin.[5,6]

SA resin cements mostly lie in the dual-cured category, combining the advantages of self- and light-cured materials. A significant attenuation of light occurs while transmitting through large and opaque restorations. Consequently, insufficient light is delivered to the cement. Hence, the polymerization of dual-cure cements depends mainly on their chemical-curing component.[7] Meanwhile, dual-cure cements attain instant and superior polymerization by light-activation at the restoration margins, which is essential for the early retention and stability of the restoration. Moreover, prompt polymerization of the cement at the restoration margins can protect its deeper and internal layers from the oral environment until the slow chemical polymerization is complete.[8,9]

Several studies investigated the polymerization kinetics of dual-curing cements and reported conflicting results regarding the impact of time at which the light is applied on the cement properties.[8,10] Although some dual-cured cements attained similar polymerization with and without light-curing, others were negatively affected by insufficient light exposure.[11,12] One study reported that immediate light-curing enhances the degree of polymerization and reduces water sorption of the cement.[13] In contrast, another study concluded that a two-minute delay in photo-activation enhanced the luting agent's conversion.[8] Other studies reported that a delay of photo-activation up to five minutes post-mixing did not jeopardize the degree of conversion while successfully reducing the polymerization stresses and increasing the bonding efficiency of the cement to the dentin substrate.[14,15]

The SA cements are hydrophilic in nature and depend on the presence of acid-functionalized monomers to demineralize the tooth surface and chemically bond to the calcium of hydroxyapatite (HAP). Therefore, sufficient time is required for the ionization of these functional monomers. It was reported that immediate photo-activation might turn the resin cement more hydrophobic; hence, delaying the photo-activation may allow a better chemical reaction with the wet dentin substrate.[16,17] Yet, the effect of delayed photo-activation on the bonding performance is material-dependent, due to the difference in the cements' chemical composition, their mechanism of bonding to dentin, and the time needed for the acidic monomers to complete their chemical reaction with the tooth substrate.[18] Nevertheless, dentin over-wetness and prolonged fluid movement through the bonded interface may hinder optimal resin seal and can jeopardize the cement's cohesive strength due to excessive water sorption.[19,20]

In the current literature, there is a lack of studies considering the effect of simulated pulpal pressure on the bonding efficacy of SA resin cements, when light-cured immediately or after a short delay. Thus, this study was conducted to assess the impact of immediate

and delayed photo-activation on the water uptake by two different dual-cured SA resin cements and their shear bond strength (SBS) to dentin under simulated pulpal pressure. The null hypothesis was that photo-activation timing (immediate or delayed) does not affect the bond strength to dentin or the water uptake of dual-cured SA resin cements under simulated pulpal pressure.

METHODS AND MATERIALS

Sample Preparation and Simulated Pulpal Pressure (SPP) Setup

Sixty-six extracted human third molars were collected from the oral surgery department at the University of Sharjah Dental Hospital after the university's research ethics committee's approval and patient consent. Teeth that were endodontically treated, carious, prepared, or bleached were excluded from the study. The teeth were cleaned from any soft-tissue remnants using an ultrasonic scaler, then kept in a 1% thymol solution in a refrigerator for one month until used in the study.

The specimens were randomly divided into two equal groups, thirty-three-teeth each, according to the SA resin cement used. The cements used in this study and their composition are summarized in Table 1. Each group was then subdivided according to the time elapsed between the cement and photo-activation application into three equal subgroups.

Each specimen was sectioned transversely using a low-speed precision diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water-cooling to remove the occlusal enamel and expose a flat dentin surface.

The roots of the teeth were then sectioned 1 mm apical to the cementoenamel junction and pulpal tissues were removed using a barbed broach (Dentsply Sirona, Tulsa, OK, USA). The remaining dentin thickness was measured using a pincer-type caliper from the outer dentin surface to the highest pulpal horn, and it was standardized for all samples to be 0.8 ± 0.1 mm. The pulp chamber was rinsed with a 2.5% sodium hypochlorite solution for 20 seconds and 17% EDTA-solution (pH 8) for 15 seconds, followed by rinsing with distilled water for another 30 seconds to neutralize the effect of the irrigation solutions. Afterwards, the pulp chamber's opening was blocked with red wax and the specimens were embedded in cold-cure acrylic resin in a cube-shaped mold (2 cm edge length). The pulp chamber was exposed by drilling a circular opening (2.5 mm in diameter) on the mold's bottom side, and the wax was removed using a hot instrument. Specimens were then kept in distilled water at room temperature to prevent dehydration.

Under water-cooled, 600-grit SiC abrasive papers were used in a polisher/grinder (EcoMet 30, Buehler) to create a uniform smear layer on the dentin surfaces. The specimens were then rinsed with double-distilled water, cleaned in an ultrasonic water bath, and kept hydrated in containers filled with double-distilled water. A Tygon tube with a 2.5-mm external diameter and 1.5-mm internal diameter (Sigma-Aldrich, Buchs, Switzerland) was inserted in the pulp chamber opening and secured with cyanoacrylate super glue (Scotch Super Glue Liquid, 3M, Maplewood, MN, USA) to seal any gap between the tube and the specimen surface. Care was taken to avoid filling the pulp chamber with glue. A 20-mL syringe was filled with deionized water and connected to the Tygon tube and was suspended 20 cm above the sample's level to simulate 20-cm H_2O pulpal pressure.

Cementation and Shear Bond Strength (SBS) Test

Eight samples from each sub-group were used for the SBS test. Pre-polymerized resin nano-composite cylinders (Filtek Z350 XT Universal Restorative, 3M Oral Care, St Paul, MN, USA) were prepared using a Teflon mold (2.0 mm in diameter and 2.0 mm in height).

Table 1: *Self-adhesive Resin Cements Evaluated: Their Composition and Respective Manufacturer's Information*

Cement	Abbreviation	Manufacturer	Composition	LOT
RelyX Unicem 2 Automix	RU	3M Oral Care, St Paul, MN, USA	Base paste: phosphoric acid-modified methacrylate monomers, bifunctional methacrylate acetate, initiators, stabilizers, glass fillers, silica, calcium hydroxide Catalyst paste: methacrylate monomers	430451
Maxcem Elite	MC	Kerr Dental Orange, CA, USA	GPDM, methacrylate ester monomers, HEMA, 4-methoxyphenol, cumene hydroperoxide, titanium dioxide and pigments	3441545

Abbreviations: GPD, glycerol phosphate dimethacrylate; HEMA, 2-hydroxyethylmethacrylate.

For all groups, the dentin surface was gently dried with a stream of air. Then the cylinders were cemented to the dentin surface using either RelyX Unicem 2 Automix (RU, 3M Oral Care) or Maxcem Elite (MC, Kerr Dental, Orange, CA, USA). For both cements, the cement base and catalyst were mixed using an auto-mixing tip and applied using an ultrafine 1-mm tip. The cements were applied on the dentin surfaces and the composite cylinders were placed over the cement layer at the center of the dentin surfaces. During the cementation of each resin composite cylinder, a special metal clip was used to stabilize the cylinder and induce a standardized load for 20 seconds. The excess cement was removed around the margins using a microbrush. From the beginning of the mixing until the complete removal of the excess cement, the total placement time was set to 40 seconds per specimen. The metal clip was removed carefully, and each sub-group (n=8) received one of the three following light-curing protocols: 1) Immediate (IM) – the cement was immediately light-cured after placement; 2) Delayed 30 seconds (D30) – the cement was light-cured 30 seconds after placement; or 3) Delayed 120 seconds (D120) – the cement was light-cured 120 seconds after placement.

Photo-activation was done from the top surface of the resin cylinders for 20 seconds using an LED light-curing unit (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) operating at 1200 mW/cm^2 in standard mode. The intensity was verified every eight samples using a digital radiometer (Cure Rite, Dentsply, Milford, DE, USA) to ensure uniform curing. After the cementation procedures, all specimens were stored in distilled water at 37°C for 24 hours to allow for cement maturation. Then, the SBS was tested using a table-top shear bond tester (Bisco Inc, Schaumburg, IL). Shear forces were applied on the tooth-restoration interface by a semicircular metal attachment that ran at 1 mm/min until complete failure of the cement and debonding. The force at the time of failure was recorded, and the bond strength was calculated by dividing the force at failure by the surface area of the bonded interface to get SBS in MPa using the following equation:

$$\text{SBS (MPa)} = \text{Force (N)} / \text{Area (mm}^2\text{)}$$

The specimens were then examined under a stereomicroscope (Model SM-3BX-80S, AmScope, Irvine, CA, USA) with magnification from 3.5X – 4.5X to determine the type of failure: whether cohesive failure within the cement (type I), an adhesive failure between the cement and the tooth (type II), or a mixed failure (type III).

Confocal Laser Scanning Microscopy (CLSM) Analysis

Three additional samples from each experimental sub-group were used for CLSM analysis. A 0.1 wt% fluorescein sodium salt dye solution in deionized water (Sigma-Aldrich, St. Louis, MO, USA) was added to the simulated pulpal fluid reservoir. After preparation and cementation procedures under SPP, the remaining dye in the pulp chamber was thoroughly washed out with water. The top surfaces of the specimens were embedded in a cold-cure acrylic resin. Afterwards, the specimens were stored in distilled water for 24 hours. After storage, the specimens were sectioned longitudinally in a direction perpendicular to the bonded interface with a slow-speed diamond saw running under water-cooling (Isomet 1000, Buehler) to obtain a 1-mm–thick section from the center of the tooth. The longitudinal sections were polished using 1200-grit SiC abrasive papers and placed in an ultrasonic water bath for five minutes to remove loose debris. The sections were mounted on glass slabs, then the tooth-restoration interfaces were examined immediately with a confocal laser scanning microscope (Nikon Eclipse Ti–S, Nikon Instruments Inc, Melville, NY, USA). Microscopic images were taken for the tooth-restoration interface using 468 nm laser illumination, with up to 20x magnification. The fluorescent dye distribution within the interface and the dye uptake by the cement layer were assessed.

Statistical Analysis

The data obtained from the SBS testing were analyzed by SPSS software (IBM SPSS statistics V24.0, IBM Corp, Armonk, NY, USA). Two-way analysis of variance (ANOVA) was used to assess the effect of different photo-activation timings and cement type on the SBS of the cements tested. Tukey post-hoc test was used to detect pairwise differences among the experimental groups. A 95% confidence level was applied for all the statistical tests (α=0.05), and the power of the study was 0.90. The Chi-square test was used to compare between failure modes of the tested groups.

RESULTS

Shear Bond Strength (SBS)

Mean SBS values and standard deviations (SD) of the experimental groups are presented in Table 2. Two-way ANOVA revealed a significant effect of both photo-activation timing and the cement type on SBS to dentin (p<0.001). The results showed that, in the two tested resin cement groups, D30 resulted in a statistically significant higher mean SBS compared to IM and D120

Table 2: *Mean Shear Bond Strength Values (MPa ± Standard Deviation) of RelyX Unicem 2 Automix (RU) and Maxcem Elite (MC) After Different Dwell Times*[a]

Dwell Time	Cement Type	
	RU	MC
IM	12.3 ± 3.2 Aa	3.9 ± 1.1 Ba
D30	22.4 ± 6.4 Ab	7.6 ± 2.6 Bb
D120	13.6 ± 6.2 Aa	4.8 ± 1.6 Ba

Abbreviations: D30, delayed 30 seconds; D120, delayed 120 seconds; IM, immediate; MC, Maxim Elite; RU, RelyX Unicem 2 Automix.

[a]*Within a row, same uppercase letters show mean values with no statistically significant difference* (p>*0.05). Within a column, same lower-case superscript letters show mean values with no statistically significant difference* (p>*0.05).*

Table 3: *Failure Type Percentage of RelyX Unicem 2 Automix (RU) and Maxcem Elite (MC) After Different Dwell Times*

Material	Failure Type	Dwell Time		
		IM N (%)	D30 N (%)	D120 N (%)
RU	Cohesive	5 (62.5)	6 (75.0)	6 (75.0)
	Adhesive	0 (0)	1 (12.5)	0 (0.0)
	Mixed	3 (37.5)	1 (12.5)	2 (25.0)
MC	Cohesive	3 (37.5)	1 (12.5)	0 (0.0)
	Adhesive	3 (37.5)	6 (75.0)	6 (37.5)
	Mixed	2 (25.0)	1 (12.5)	2 (25.0)

Abbreviations: D30, delayed 30 seconds; D120, delayed 120 seconds; IM, immediate; MC, Maxim Elite; RU, RelyX Unicem 2 Automix

(p<0.05). Moreover, RU showed significantly higher SBS mean values compared to MC (p<0.05), regardless of the photo-activation timing applied.

Failure modes of the SBS tested specimens are summarized in Table 3. The Chi-square test showed that there was a statistically significant difference between failure modes of the two materials (p<0.001). Under the three photo-activation timings, the predominating type of failure was cohesive in RU groups and adhesive in MC groups.

Confocal Laser Scanning Microscopy (CLSM)

Representative images of the CLSM analysis are presented in Figure 1. Fluorescent dye uptake was noted in all tested groups. Still, its extent and manifestation varied according to the cement type and the time elapsed before photo-activation. In the IM specimens of RU, the dye uptake was confined to the cement-dentin interface and within the thin hybrid layer (Figure 1A), while the hybrid layer was thicker in the D30 group, up to 10 μm from the adhesive interface (Figure 1B). In addition, no gap formation was noticed in these two groups. In contrast, in D120, the dye uptake was more obvious, showing a structureless pattern that varied in width but was approximately 50 μm at its widest, and discrete gaps were found at the dentin-cement interface (Figure 1C).

In the MC groups, the dye uptake increased with extending the waiting time before photo-activation, and it was generally more obvious than that of RU groups. The dye uptake in the IM group was more scattered and less obvious compared to the more noticeable and uniform distribution of the dye along the adhesive interface in D30 and D120 groups (Figure 1D). Moreover, D30 showed a scattered dye infiltration through the cement layer that reached the cement-restoration interface (Figure 1E). D120 showed a highly stained thick zone invading the entire thickness of the cement layer, with complete separation at the adhesive interface (Figure 1F).

DISCUSSION

This study was conducted to evaluate the effect of the time elapsed between the placement of two dual-cured SA resin cements and light-activation on their bonding efficacy to both the dentin substrate and the restoration by assessing the SBS in the presence of SPP followed by determining the failure mode. In addition, CLSM was utilized to examine the dye uptake extension and pattern at the tooth-restoration interface. The results of this study revealed a significant effect of the photo-activation timing on the SBS of the tested cements; thus, the null hypothesis was rejected.

In the current study, the tested cements were either photo-activated immediately, after 30 seconds or after 120 seconds following their placement. The authors chose these delay periods as they considered them more clinically acceptable than those proposed by previous studies, in which the delay in photo-activation was up to 10 minutes.[8,21]

There is a great divergence of views in the literature regarding the effect of time elapsed between applying the dual-cured cements on dentin and their photo-activation. While delaying the photo-activation may not increase the polymerization potential of resin-based cement, its ability to reduce the induced polymerization stress and increase the bond strength to dentin has been shown in some studies.[21,22] A study by Fonseca

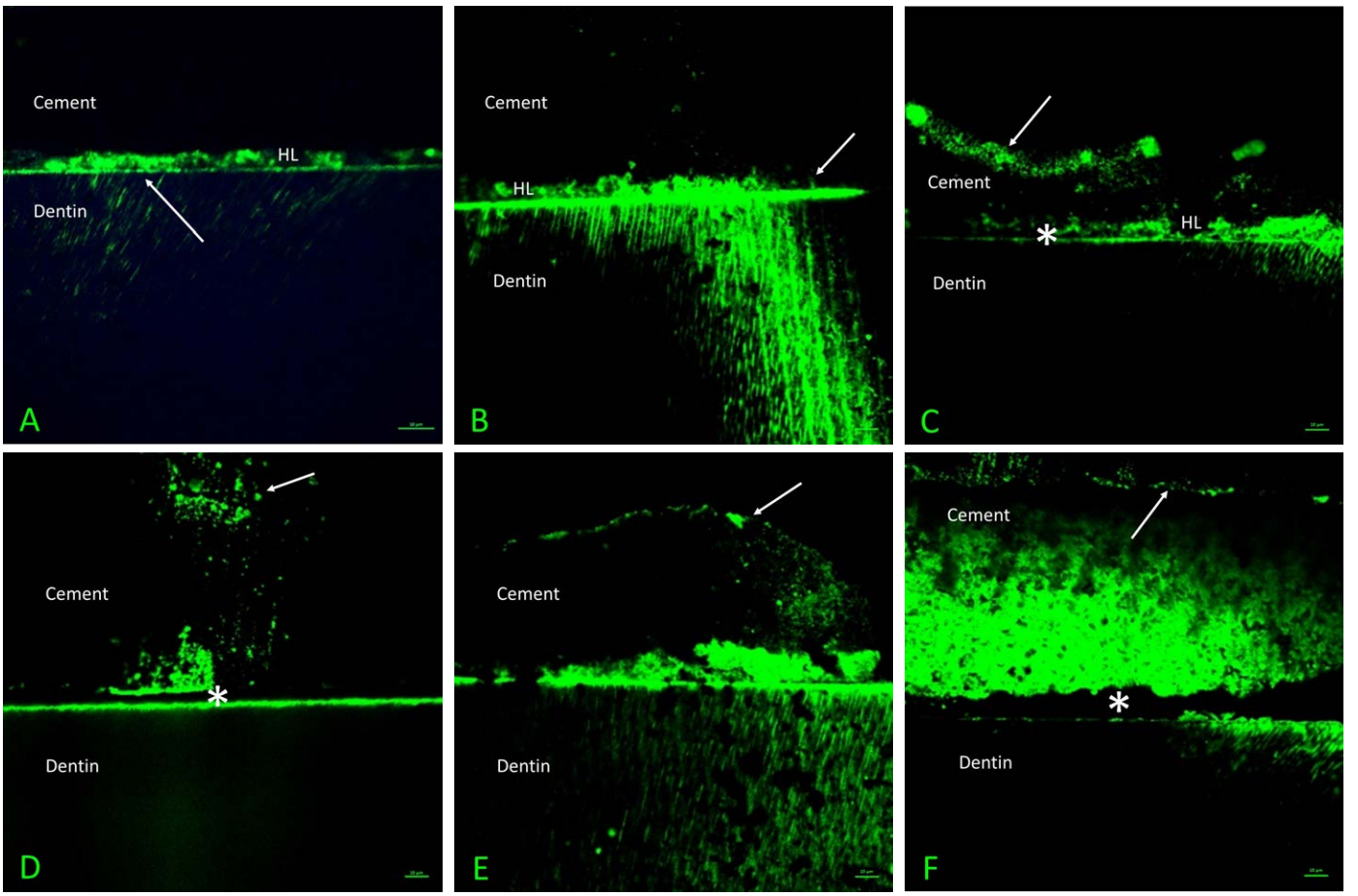

Figure 1. *Representative confocal laser scanning microscopy (CLSM) fluorescence images showing the green, fluorescent dye uptake under simulated pulpal pressure. A) RelyX Unicem 2 Automix (RU) immediate photo-activation; dye uptake within the thin hybrid layer (HL) and the dentin adhesive interface (white arrow); B) RU with 30-sec delay; dye uptake within the wider HL and the dentin adhesive interface (white arrow); C) RU 120-sec delay; a deeper dye uptake, up to 50 µm within the cement layer (white arrow) and discrete gaps at the adhesive interface (asterisk); D) Maxcem Elite (MC) Immediate photo-activation; separation at the adhesive interface (asterisk) and discrete dye uptake through the cement layer (white arrow); E) MC 30-sec delay; uniform dye distribution along the HL and reaching the top surface of the cement layer (white arrow); and F) MC 120-sec delay, severe dye uptake through the entire thickness of the cement layer and reaching to the top surface (white arrow), complete separation at the dentin-cement interface (asterisk).*

and others investigated the effect of delayed photo-activation on SA resin cement's degree of conversion and water sorption; the authors concluded that the photo-activation timing modified the behavior of the cement and that the dual-cured SA resin cements should be photo-activated as soon as possible after placement.[13] Unfortunately, in most of these studies, testing the properties of SA cements was not done under SPP; hence, the effect of dentinal fluids on the bonding efficacy of the cement was not considered.

Within the two investigated cement groups, the results of our study showed that the mean SBS was significantly higher for the D30 groups than the IM and D120 groups. Self-adhesive cements bond to the tooth tissues through a twofold mechanism: micromechanical interlocking and chemical bonding. The first is created by the acidic monomers that partially demineralize the dentin and simultaneously infiltrate the collagen network, creating a hybrid layer. While the second mechanism is slowly established through chemical bonding between the self-adhesive functional monomers and the remaining HAP around the demineralized collagen within the relatively thin hybrid layer.[23,24] According to the "adhesion–decalcification" (AD) concept that was described by Yoshida in 2004, functional monomers like 10- methacryloyloxydecyl dihydrogen phosphate (10-MDP) bond electrostatically to HAP, creating a primary chemical bond to the calcium of HAP and forming a characteristic self-assembled nano-layered structure.[25] In our study, RelyX Unicem was chosen as a representative of the MDP-based SA cements, because it is the most thoroughly investigated SA cement in the literature. Similarly, most of the previous studies done on non-MDP–based SA cement used Maxcem Elite.[26]

We postulate that a 30 second-delay in light-curing allowed the functional phosphoric acid esters, 10-MDP in RU, and glycerol dimethacrylate dihydrogen phosphate (GPDM) in MC, to sufficiently demineralize the dentin surface, optimizing the micro-mechanical adhesion. In addition, this 30-second delay of photo-activation might have permitted the functional monomers to interact with the calcium of HAP and eventually resulted in a significant nano-layering without being interrupted by photo-activation.

GPDM was utilized for a long time in etch-and-rinse adhesives, such as Optibond FL (Kerr), and exhibited high bond strength to dentin, both immediately and upon aging.[27,28] Subsequently, GPDM was added as a functional monomer to self-etch adhesives, SA cements, and SA restorations. The bonding performance of GPDM-based SA restorations was reported to be inferior to that of MDP, due to its high hydrophilicity.[28,29] these findings are consistent with our results, which revealed that RU showed significantly higher (p<0.05) SBS mean values compared to MC regardless of the photo-activation timing applied. The superior performance of RU can be attributed to its ability to form nano-layering of stable 10-MDP-Ca salts and a durable bond to HAP, compared to the reported unstable bond of GPDM to dentin.[27-31] Further explanation was described by Van Meerbeek and others, who described a submicron HAP-rich hybrid layer formation without obvious collagen exposure by the 10-MDP-based adhesives.[32,33] In contrast, a parallel study by Yoshihara and others reported that GPDM-based adhesives yielded a thicker (1.5 to 2 μm) HAP-poor hybrid layer with visible collagen exposure.[28]

Light-activation of dual-cured cements induces fast polymerization, which produces a large number of free radicals. The early vitrification by the light-activation causes the entrapment of the free radicals within the organic matrix, and hence minimizes the extent of the subsequent self-polymerization of these cements, which compromises the overall degree of conversion of the polymer.[8,34] This may explain the low bond strength values obtained from the immediately light-cured groups. This justification may suggest that delaying the light activation procedure would result in a higher degree of conversion of the cement, and consequently, a higher bond strength. This postulation may be true until a certain point is reached, when the chemical curing time becomes a limiting factor due to the cement reaching a high level of viscosity (vitrification point), restricting the mobility of the unreacted monomers.[11]

In the current study, both cements exhibited lower mean SBS values with a photo-activation delay of 120 seconds compared to those reached by a 30-second delay. These results might further emphasize the impact of reaching and/or exceeding the aforementioned limiting point in the time spectrum of complete polymerization on the bond strength. Moreover, although the acidic functional monomers are mostly consumed by the reaction with the HAP of the tooth, the remaining unreacted acidic monomers are believed to inhibit chemical polymerization by their deactivating effect on free radicals. Therefore, if insufficient polymerization is achieved by photo-initiation at a certain point, the self-curing process may be significantly compromised.[5]

It is generally described that a serum-like fluid fills the dentinal tubules and flows from the pulp chamber by hydrodynamic pressure of approximately 24 cm Hg in vital teeth. The egression of pulpal fluid has been shown to compromise the bonding of adhesive systems.[35] The effect of simulated pulpal pressure on the bonding characteristics of SA cements was confirmed by our CLSM findings, as the fluorescent dye intake generally increased with delaying the photo-activation timing. The immediate curing groups showed limited dye uptake, which was only confined to the restoration/dentin interface area (Figure 1A and 1D). In contrast, the results may indicate that if the egress of the pulpal fluids under pressure is maintained for a relatively long time, it may cause separation between the cement and the dentin substrate. Additionally, the pulpal fluid can infiltrate through the entire thickness of the cement layer and may reach the cement-restoration interface, as shown at the adhesive interface of the D120 groups of MC (Figure 1F).

The prolonged contact between the uncured hydrophilic monomers and the dentin surface may increase the dentinal fluid uptake within the adhesive interface, negatively influencing the cement polymerization and bonding efficacy.[36] This effect might explain the lower bond strength values and a higher percentage of cohesive failures within the D120 groups.

Our study's findings are in agreement with those reported by Hiraishi and others, who concluded that the pulpal fluid permeation during the initial setting period could deteriorate the bonding quality of resin cement. This deteriorating effect was signified as reduced microtensile bond strengths and porous bonding interfaces through the cured adhesive layer.[17] In contrast, Mazzitelli and others indicated that the SPP effect is material-dependent; while some SA cements, namely RU and Bis-Cem (Bisco), had a significant increase in their bond strength under SPP, other cements were not significantly influenced by the presence or absence of SPP. The authors explained that the lack of water in the RU composition justified

the essential role of the water from the pulpal fluid and wet dentin in ionization of the phosphoric acid esters, which enable the phosphoric acid to react with the alkaline fillers and tooth apatite, leading to better setting and improved bonding.[16]

The predominate type of failure in RU groups was cohesive in nature. The authors cannot attribute these results to the materials' immoderate water uptake, as the CLSM analysis revealed lower water uptake by RU compared to MC. Alternatively, these results can be credited to the established strong adhesive bond with the dentin substrate, which may have surpassed the cohesive strength of the cement. Similar results were reported by Mazzitelli and others, who showed a higher percentage of cohesive failures within the SA cements' layers under SPP than when no SPP was applied.[16] Another study showed that under SPP, the predominant type of failure noticed in RU was cohesive, while the highest percentage of failure in all other investigated cements was adhesive.[17]

One limitation of the current study is that it assessed the immediate bond strength of the tested materials (after 24 hours storage). Although the results of our study may be predictive of the long-term performance of the tested SA cement under deteriorating oral environmental conditions, aging the samples under wet storage conditions, alone or with thermal and/or mechanical loading, could have given more clinically relevant information.

CONCLUSION

Under SPP testing conditions, it can be concluded that a 30-second delay after the placement of the SA cement on dentin is recommended before photo-activation. This time is sufficient for the SA functional monomers to complete their chemical reaction with dentin. Simultaneously, this delay period is short enough to avoid the pulpal fluid's deteriorating effect associated with the prolonged delay of photo-activation. Compared to GPDM-based cements, MDP-based cement showed superior bond strength and less pulpal fluid uptake, regardless of the photo-activation delay time applied.

Regulatory Statement

This study was conducted in accordance with all provisions of the human subjects oversight committee guidelines and policies of the University of Sharjah Research Ethics Committee (approval code REC-18-10-09-03-S).

Conflict of Interest

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

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Comparison of the Mechanical Properties and Push-out Bond Strength of Self-adhesive and Conventional Resin Cements on Fiber Post Cementation

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Clinical Relevance

The mechanical behavior of self-adhesive resin cements is not superior to conventional resin cements.

SUMMARY

Objectives: The purpose of this study was to compare the mechanical properties and push-out bond strength of self-adhesive resin cements (SACs) and a conventional resin cement (CRC).

Methods and Materials: Eighty bovine incisors were divided into four groups for cementation of a fiberglass post (Whitepost - FGM Dental Group, Coral Springs, FL) with different resin cements: three SACs (Maxcem Elite, MAX - Kerr; Calibra Universal, CAL - Dentsply; and RelyX Unicem 2, RUN - 3M Oral Care) and one CRC (RelyX Ultimate, RXU - 3M Oral Care). The groups were subdivided into two groups each (n=10) for evaluation of the push-out bond strength test (POBS) after 24 hours of water storage or after thermal aging (5000 cycles), following 24 hours of storage. The failure modes were evaluated using a stereomicroscope. Flexural strength (FS) and modulus of elasticity (EM) were determined using a three-point bending. Also, pH of the cements

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was measured over 48 hours and filler morphology was observed by scanning electron microscopy. Appropriate statistical analyses were performed by SPSS 21.0 (SPSS Inc., Chicago, IL, USA), with a significance level set at 5%. Results: RXU presented the highest POBS at both evaluation times. Among the SACs, RUN and CAL presented significantly lower POBS than MAX in cervical and middle-thirds at the 24-hour evaluation, and in all root regions after thermocycling. Adhesive failure between the cement and dentin were the most prevalent fractures at both times evaluated. MAX presented the lowest FS and RUN showed the highest EM. The pH reached the minimal point at the 30-minute evaluation for RXU and MAX. For RUN and CAL, the minimal pH was observed at the 60-minute evaluation. RXU and RUN presented spherical and regular filler particles, while MAX and CAL presented irregularly shaped and sized filler particles.

Conclusions: The mechanical behavior of SACs is not superior to CRC; however, among all the SACs evaluated, MAX presented the highest POBS and stability after thermocycling evaluation. MAX also reached the closest neutral pH after 48 hours. Therefore, SACs with low initial pH and strong neutralization reactions are recommended, because these characteristics may lead to better mechanical properties and stability.

INTRODUCTION

The luting procedure is one of several factors that lead to clinical success of fiberglass post (FRC) cementation.[1,2,3] Conventional resin cements (CRC) are the usual material of choice because of their good mechanical properties.[4,5] However, CRCs are technique sensitive because of the numerous steps involved, such as acid etching the root canal, and the application of an adhesive system. These steps can be affected by operator mistakes, causing misplacement of the FRC.[6,7,8] In addition, adhesion into the radicular dentin is challenging because of polymerization shrinkage stresses resulting from a high configuration factor, and the limited access and visibility.[2,3] Hence, in response to clinical concerns, a new kind of self-adhesive resin cement (SAC) was developed to simplify the luting procedure and promote good adhesion in clinical situations where there is low mechanical retention.

Commercially available, SACs adhere to tooth structure without intermediary adhesives or etchants, combining ease of application with mechanical and adhesive properties similar or higher to those of CRCs.[1,8] Nonetheless, besides the mentioned simplified technique, the main difference between SACs and CRCs are the composition and bonding mechanism to the dental structure.[3,8,9] In short, traditional SACs include acidic functional monomers to promote etching of the mineralized tissues,[8,9] a dual cure polymerization mechanism, and fillers that neutralize the low pH of the cement.[7,8]

Adhesion by SACs occurs when the acid-functionalized monomers such as methacrylate with carboxylic or phosphoric acid-groups etch the enamel and dentin, while also producing a chemical bonding to the calcium on hydroxyapatite of the substrate.[9,10] Based on this mechanism, it is important to analyze the changes of the pH of the cements during setting and curing, because high hydrophilicity leads to water sorption, degradation, and consequently compromises the mechanical stability of the material.[9,10,11] Another potential effect of cement on the outcome of restorations is related to the composition of each resin cement, which can affect the flexural strength (FS) and elastic modulus, which in turn are indicators of the ability to resist stresses without deformation or fracture.[10-13]

Recently a review about the current status of clinical studies on SACs found that only a few studies compared the performance of SACs with that of CRCs, and none of the studies reported retention loss for either cement type.[14] However, there is scarce literature on the behavior of SACs, and how the differences of the mechanical properties produced by the alteration in the composition of a SAC compared to a CRC affect the performance of this material.[8] Hence, the comparative analysis of SACs and CRCs becomes clinically relevant and can provide important information to clinicians, by characterizing the behavior of the cements.[4,11,14]

Thus, the aim of this study was to evaluate the push-out bond strength (POBS) of fiberglass posts cemented with SACs and CRCs to root dentin after 24 hours and after thermocycling, and to compare the flexural strength, modulus of elasticity, pH-neutralization behavior, and filler morphology. The null hypotheses were: 1) the POBS of the SACs would not be different to that of the CRCs; 2) the POBS of the evaluated cements would not change after thermocycling; 3) there would not be differences on the flexural strength and modulus of elasticity of the evaluated cements; and 4) the pH of the evaluated cements would not change during the setting reaction.

METHODS AND MATERIALS

Four resin cements were included in the study: three SACs (Calibra Universal [CAL], Dentsply Sirona,York,

PA, USA; MaxCem Elite [MAX], Kerr, Orange, CA, USA; and RelyX Unicem 2 [RUN], 3M Oral Care, St Paul, MN, USA) and one dual cure CRC RelyX Ultimate 2 (3M Oral Care), as described in Table 1.

Push-Out Bond Strength Test

For POBS evaluation, the procedure was validated, and the required sample size was calculated based on a pilot study. Bovine incisors with closed apex, were used for POBS evaluation. The crowns of the teeth were sectioned 2 mm below the cement-enamel junction, and root canals with a remaining length between 16 and 18 mm were selected for use in the study, to standardize the working length at 15 mm (±1 mm). Then, the thickness of the circumferential coronal dentin was measured at four positions (mesial, distal, buccal, and lingual) with a digital caliper (Mitutoyo, Suzano, SP, Brazil).[15] The root canals with a coronal dentin thickness of 2 mm (±0.5 mm) were selected. The process was repeated until 80 specimens that complied with the established criteria were obtained. The selected root canals were divided into four groups for POBS evaluation of each cement. The groups were further subdivided into two groups for each cement (n=10), one for POBS evaluation 24 hours after specimen preparation, and

Table 1: *Commercial Name, Classification, Curing Time and Composition of the Tested Materials*

Material (lot number)	Classification	Light Cure Time (seconds for each surface)	Composition
RelyX Ultimate - 3M ESPE, St Paul, MN, USA (#6018295)	CRC	40	Base paste: methacrylate monomers containing phosphoric acid groups, methacrylate monomers. Catalyst paste: methacrylate monomers. Fillers (43 vol%): Silanated fillers; Alkaline (basic) fillers; Initiators: Sodium toluene-4-sulphinate, disodium peroxodisulphate, Tertbutyl 3,5,5 Trimethylperoxyhexanoate
RelyX Unicem 2 - 3M Oral Care, St Paul, MN, USA (#3579029)	SAC	20	Base Paste: Silane-treated glass powder, 2-Propenoic acid, 2- methacryl-oxyethyl phenyl hydrogen phosphate (Phenyl-P), 2- methyl, 1,10-[1-(hydroxymethyl)-1,2-ethanodiyl] ester, triethylene glycol dimethacrylate (TEGDMA), silane-treated silica, glass fiber, sodium persulfate, and Tert-butyl peroxy-3,5,5-trimethylhexanoate. Catalyzer paste (43 vol%): Silane-treated glass powder, dimethacrylate substitute, silane-treated silica, Sodium p-toluenesulfonate, 1-Benzyl-5-phenylbarbituric acid, calcium salts, 1,12-Dodecanediol dimethacrylate, calcium hudroxide, and titanium dioxide.
Calibra Universal - Dentsply Sirona, York, PA, USA (#180108)	SAC	10	Urethane Dimethacrylate; Di- and Tri-Methacrylate resins; Phosphoric acid modified acrylate resin (Dipentaerythritol penta-acrylate monophosphate: PENTA-P); Barium Boron fluoroaluminosilicate Glass; Organic Peroxide Initiator; Camphorquinone (CQ) Photoinitiator; Phosphene Oxide Photoinitiator; Accelerators; Butylated Hydroxy Toluene; UV Stabilizer; Titanium Dioxide; Iron Oxide; Hydrophobic Amorphous Silicon Dioxide Particles of inorganic filler range from 16 nm to 7 µm, average particle size 3.8 µm, total filler 48.7% by volume.
MaxCem Elite - Kerr, Orange, CA, USA (#7293230)	SAC	10	Multifunctional DMAs, GPDM, proprietary Redox initiators and photo-initiators, barium, fluoroaluminosilicate, and fumed silica (46% by volume)

Abbreviations: CRC, conventional resin cement; GPDM, glycero-phosphate dimethacrylate; SAC, self-adhesive resin cement

the other group was tested after thermocycling aging following 24 hours of storage.

Cleaning and shaping of root canals were performed by crown-down technique, using K-type files #80 (Dentsply Maillefer, Ballaigues, Switzerland). Working length was set 1 mm short of the apical foramen, based on the clinical length of the roots. Apical enlargement was performed manually using a #70 file. Afterward, the specimens were washed with 0.5% sodium hypochlorite solution and 17% EDTA for 1 minute each. Sealing of the root canals was performed by lateral condensation of gutta percha cones (Dentsply Sirona, York, PA, USA), using a calcium hydroxide cement (Sealer 26; Dentsply Sirona). The coronal access of the specimens was sealed with Coltosol (Coltene, Rio de Janeiro, RJ, Brazil) and stored at relative humidity for seven days in an oven (Fanen, Guarulhos, SP, Brazil) at 37°C.[15] After storage, 12 mm (±1 mm) of intracanal gutta percha were removed using the specific bur of the selected glass-fiber post system (White Post DC #3, FGM, Joinville, SC, Brazil) in a low-speed handpiece (Kavo, Kerr, SP, Brazil) in a single perpendicular movement. After preparation, the root canals were rinsed with distilled water to remove remaining gutta percha and dried using absorbent paper tips (Dentsply Maillefer).[15] Prior to cementation, the fiber post was cleaned with 70% alcohol according to manufacturer's instructions.

Resin Cement Luting—For the RXU, a drop of a one-step adhesive (Single Bond Universal, 3M Oral Care) was applied on the FRC post surface and in the canal wall according to manufacturer's recommendation. For SACs, the fiber post and the canal wall did not receive any pretreatment, according to the manufacturer's instructions. For the CRC, the cement was mixed and inserted into the canal using the intracanal needle tip of a Centrix system (DFL; Rio de Janeiro, RJ, Brazil). For CAL, RUN, and MAX, the cements were applied using the self-mixing tip supplied by the manufacturers. In all groups, the FRC was placed into the root canal and while excess cement was removed, a digital pressure was applied for one minute. The cements were light cured according to the manufacturers' recommended time at 0 mm distance, using a light curing unit (Valo, Ultradent Products Inc; South Jordan, UT, USA) with a radiant emittance of 1200 mW/cm². Classification, brand name, composition, and exposure time of the evaluated materials are presented in Table 1. A schematic representation of the configuration of the root canal after the luting procedure is presented in Figure 1.

The specimens were stored in a dark oven for 24 hours in 100% relative humidity at 36°C. After storage, specimens were sectioned using a slow-speed cutting machine (Isomet 1000, Buehler, Uzwil, Switzerland) into

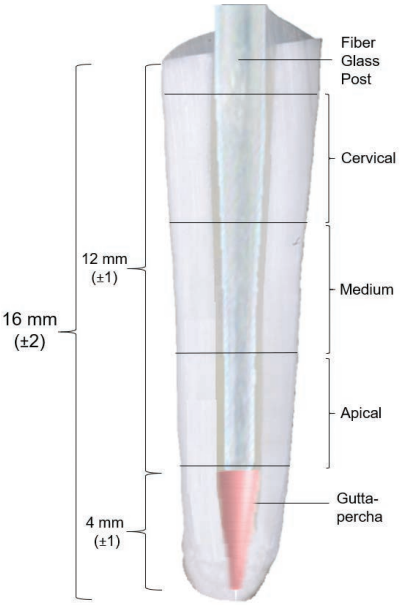

Figure 1. *Schematic representation of the fiberglass post cementation and the slices obtained for the Push-Out Bond Strength.*

three, 1-mm-thick slices from each radicular third.[16,17] The thickness of the obtained slices was measured and registered, and the POBS test (n=10) was realized. For the group treated by thermocycling aging following 24 hours of water storage, in the cycling machine (MCT2-AMM2, São Paulo, SP, Brazil), the specimens were submitted to 5000 cycles in water at 5°C (±1°C) to 55°C (±1°C), 30 seconds at each temperature and a transfer time of five seconds, simulating six months of aging. After thermal cycling, the roots were sliced following the aforementioned protocol and the POBS was measured.[18] The POBS evaluation (n=10) was performed using a universal testing machine (Instron, Norwood, MA, USA) at 1.0 mm/min of speed.[16]

Failure Mode

After POBS evaluation, the debonded specimens were recovered and observed using a stereomicroscope (Carl Zeiss Inc, Oberkochen, Germany) at 40× to categorize the failure mode. The observed patterns were: (I) adhesive failure between the cement and the dentin; (II) adhesive failure between the cement and the post; (III) cohesive failure in the cement; (IV) cohesive failure in the post; and (V) mixed failures consisting in a combination of two or more failure modes.[17,18]

Flexural Strength and Modulus of Elasticity

For flexural strength and modulus of elasticity measurement, the specimens (n=15) were prepared according to ISO 4049. The specimens were molded

using a customized mold (2×2×25 mm), light cured (Valo, Ultradent Products, Inc) and finished using a 1200-grit abrasive paper to remove flanges. After finishing, the specimens were stored in water for 24 hours at a temperature of 36°C.[19,20,21] The bars were fixed in a three-point-bending test rig (fin distance 20 mm) of a universal testing machine (Instron-Norwood, MA, USA) and loaded until fracture with a crosshead speed of 1.0 mm/min.

pH Measurement

The resin cements (n=3) were filled into a silicone mold (diameter 20 mm, thickness 2 mm) and a Mylar strip was positioned on the top of the surface to prevent the formation of an air inhibition layer. The specimens were light cured according to manufacturer's recommendation. Afterwards, the samples were individually immersed in 10 mL of distilled water with a pH-electrode (An2000, Analion, Ribeirão Preto, SP, Brazil) on the top, and evaluated over 1-, 5-, 15-, 30-, 60-minutes, 24 hours and 48 hours, protected from light to exclude any curing influence.[20] After each pH evaluation, the distilled water was changed, measured (baseline pH=6) and the pH electrode was recalibrated at pH 4 and pH 7.

Filler Morphology

For each material, 60 mg of unpolymerized cement paste was placed in a plastic tube (n=1). The unpolymerized cements were dissolved in 6 mL of acetone (99.5%, Merck KGaA, Darmstadt, Germany) and subjected to agitation for 1 minute. After mixing, the obtained suspension was centrifuged for 5 minutes at 14,000 rpm, and the supernatant was discarded. Then, 6 mL of chloroform 99.8%, (Merck KGaA) was used to redisperse the sediment, and the mixing and centrifugation procedures were repeated. The supernatant was discarded, and the remaining sediment of filler particles was redispersed in 6 mL of absolute ethanol (Merck KGaA). Three 20-μL drops of the obtained suspension were placed in a metallic stub and covered in a desiccator for 24 hours.[22] The stubs were sputter-coated with gold in a vacuum evaporator (MED 010, Bal-Tec, Balzer, Liechtenstein) and observed using a scanning electron microscope (SEM) (JSM-5600, JEOL Inc, Peabody, USA) at 1000× magnification.

Statistical Analysis

Data were tested for normality and homoscedasticity (Shapiro-Wilk and Levene test). The POBS was analyzed by three-way analysis of variance (ANOVA, factors: cement, root region, and evaluation time) and Bonferroni *post hoc* test (α=0.05). Flexural strength and modulus of elasticity were tested by one-way ANOVA and Tukey *post hoc* test for resin cement comparison. The pH was analyzed by one-way repeated measures ANOVA (factor cement) and Bonferroni *post hoc* test (α=0.05). Failure mode data were submitted to the Pearson chi-square statistical test. Analyzes were performed by SPSS 21.0 (SPSS Inc., Chicago, IL, USA), with a significance level set at 5%.

RESULTS

Push-out Bond Strength Test

The mean POBS values of all the evaluated cements are presented in Table 2. The ANOVA for POBS found that the resin cement (p<0.001), root region (p<0.001) and evaluation time (p<0.001) significantly influenced the results, as well as the triple interaction between all factors (p=0.025). Comparison between cements showed that the POBS of the CRC-RXU was higher than the SACs, in all radicular regions and evaluation times (p<0.024), except for MAX in cervical and middle thirds at the 24-hour evaluation, and at the cervical region after thermocycling (p>0.05). Among the SACs, RUN and CAL presented lower POBS than MAX in the cervical and middle thirds at the 24-hour evaluation, and in all root regions after thermocycling (p<0.048). In general, the POBS at the apical third was lower than at the cervical region (p<0.038), except for CAL and RUN after thermocycling. Thermocycling produced a reduction on the POBS of all the cements and radicular thirds (p<0.048), except for CAL and RXU in the middle and cervical root regions, respectively.

Failure Mode

The frequency of failure mode distribution is represented in Figure 2. The Pearson chi-square statistical test indicated that the association between resin cement and failure mode distribution with statistical differences [$x^2(42) = 129.87$; p<0.001].

There was a lower occurrence (adjusted residual < -1.96) for the type of failure within the following groups: in 24-hour, type I and III (RXU type I [RUN], type II [CAL], and type V [MAX]); and after thermocycling, type II (RXU), type II and V (RUN), type II (CAL), and type V (MAX). On the other hand, there was a greater occurrence (adjusted residual > 1.96) for the failure type within the groups: in 24-hour, type II and V (RXU), type III (CAL), and type II and III (MAX); and after thermal cycling, type I (RXU), type I (RUN), and type I (MAX).

Table 2: *Mean (SD) Push-out Bond Strength (MPa) of the Evaluated Resin Cements According to Evaluation Time and Root Region*[a]

Type of Cement	24 Hours			Thermocycling (5000 cycles following 24 hours storage		
	Cervical	Middle	Apical	Cervical	Middle	Apical
RXU	89.96 (18.7) Aa	79.67 (7.6) ABa	69.93 (15.1) Ba	84.08 (12.7) Aa	54.55 (15.7) Ba*	55.74 (10.1) Ba*
RUN	69.13 (14.9) Ab	53.15 (14.2) Bb	52.20 (7.5) Bb	24.55 (9.7) Ac*	20.00 (4.6) Ac*	16.20 (3.3) Ac*
MAX	78.91 (18.5) Aa	78.74 (25.1) Aa	64.37 (9.1) Bb	53.90 (11.9) Aa*	41.14 (11.08) Bb*	47.37 (15.6) ABb*
CAL	44.92 (15.5) Ab	32.56 (11.5) Bc	41.80 (13.4) ABc	27.96 (8.9) Ab*	29.42 (13.8) Ac	30.05 (8.9) Ac*

Abbreviations: CAL, Calibra Universal; MAX, Maxcem Elite; RUN, RelyX Unicem; RXU, RelyX Ultimate.

[a] *Means followed by similar characters indicate no significant difference. Uppercase letters compare radicular thirds within the same cement and evaluation time (rows). Lowercase letters compare cements within the same root region and evaluation time (columns).*

* *Indicates significant differences between evaluation times for the same root region and cement.*

Flexural Strength and Modulus of Elasticity

The mean flexural strength (MPa) and modulus of elasticity (GPa) values are presented in Figure 3A and 3B, respectively. MAX cement presented the lowest flexural strength among all cements ($p<0.001$) and there were no significant differences between CAL, RUN, and RXU. RUN showed the highest modulus of elasticity of all cements ($p<0.001$), followed by RXU. There were no significant differences between MAX and CAL.

pH Neutralization

The pH profiles of all tested materials are presented in Figure 4. The one-way repeated measures ANOVA for pH detected significant influence of the material (Table 3). All resin cements presented an initial decrease of the pH that reached the minimal point at the 30-minute evaluation for RXU and MAX. For RUN and CAL, the minimal pH was observed at the 60-minute evaluation. However, for all the cements, the pH started to increase after reaching the minimal point, and at the 48-hour evaluation, the pH was equal or above the initial measurement.

Filler Morphology

The SEM images show that there are notable differences in the filler particles' size and shape

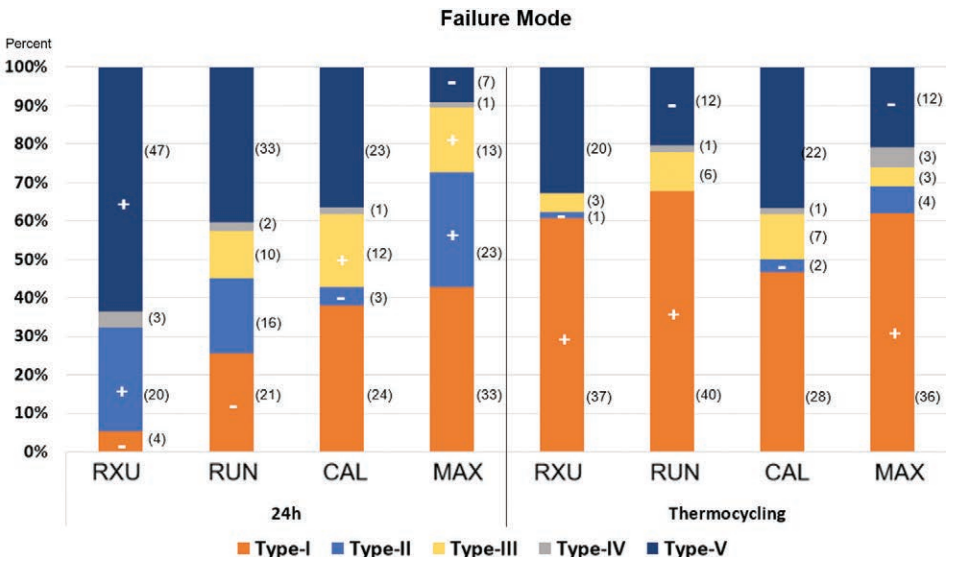

Figure 2. *Distribution of failure mode (%) of the resin cements at 24 hours and after thermocycling followed by 24 hours storage. (+) represents higher occurrence of each failure mode in each resin cement; and (-) represents lower occurrence of each failure mode in each resin cement. (#) represents the number of occurrences in percentage. Failure mode: (I) adhesive failure between the cement and the dentin; (II) adhesive failure between the cement and the post; (III) cohesive failure in the cement; (IV) cohesive failure in the post; and (V) mixed failures consisting of a combination of two or more failure modes. Abbreviations: CAL, Calibra Universal; MAX, Maxcem Elite; RUN, RelyX Unicem; RXU, RelyX Ultimate.*

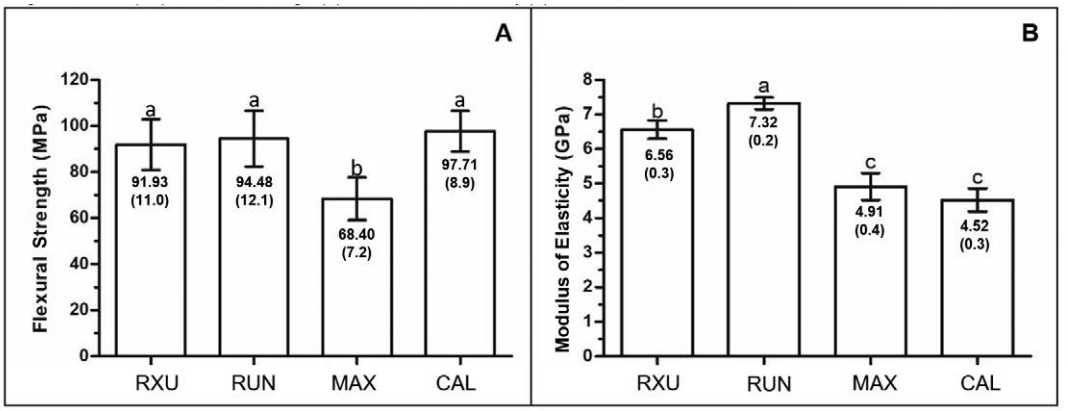

Figure 3. *Means (SD) for flexural strength (A) and modulus of elasticity (B) of the resin cements tested. Different letters indicate significant differences according to one-way ANOVA and Tukey post hoc test for resin cement comparison (α=0.05). Abbreviations: CAL, Calibra Universal; MAX, Maxcem Elite; RUN, RelyX Unicem; RXU, RelyX Ultimate.*

between the different cements. For RXU and RUN, the filler particles show similar sizes and shapes (Figure 5A and 5B), although there are evident differences in comparison with MAX and CAL (Figure 5C and 5D). For RXU and RUN, the filler particles are somewhat spherical and show a regular size, while for MAX and CAL, the fillers present irregular shapes and sizes.

DISCUSSION

The POBS results suggest that using a CRC for post cementation produces a higher bond strength than the evaluated SACs. Considering that, the first null hypothesis, that the POBS of the SACs would not be different from the CRC, was rejected. Despite previous studies indicating that SACs exhibit better mechanical properties for fiberglass post cementation and increase the micromechanical retention and chemical bonding while also exhibiting a greater tolerance to humidity and lower polymerization shrinkage stress compared to CRCs, those claims are not in concordance with the findings of this study.[23,24,25] The SACs are composed of two main components, the conventional monomers (methacrylates) and acid monomers (monomers with carboxylic or phosphoric acid-groups).[8,9] The acidic monomers etch the dental tissues; however, they have lower capacity for demineralization compared to the traditional etch-and-rinse technique, leading to lower hybridization, which compromises the mechanism of adhesion when compared to the CRC approach where the etching and bond are separated.[8,9,26]

Along with the challenges of the self-etching, especially for intraradicular post cementation, the smear layer in the root canal is denser than that of a cavity preparation on coronal dentin, and the viscosity of the cements may compromise the effectiveness of

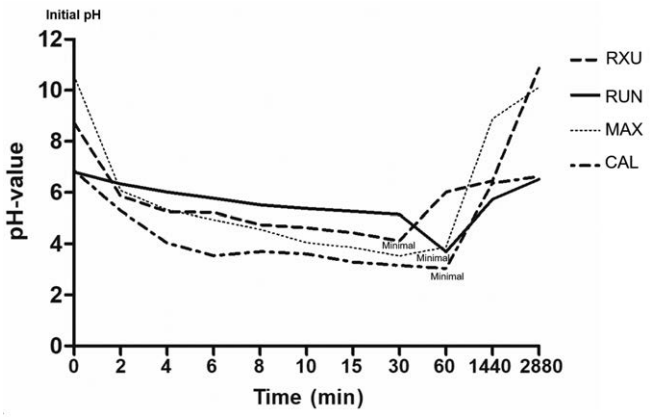

Figure 4. *pH profiles of the resin cements over 48-hour period. Abbreviations: CAL, Calibra Universal; MAX, Maxcem Elite; RUN, RelyX Unicem; RXU, RelyX Ultimate.*

Table 3: *Mean and Standard Deviation of pH Value*[a]

Type of Cement	Time Measured		
	Minimal	24 Hours	48 Hours
RXU	4.11 (0.11) C	6.45 (0.20) B	10.87 (0.31) A
RUN	3.69 (0.30) B	5.73 (1.24) AB	6.52 (0.33) A
MAX	3.53 (0.39) B	8.87 (1.74) A	10.11 (1.73) A
CAL	3.03 (0.04) B	6.36 (0.26) A	6.61 (0.19) A

Abbreviations: CAL, Calibra Universal; MAX, Maxcem Elite; RUN, RelyX Unicem; RXU, RelyX Ultimate.

[a]*Mean values represented with different letters are significantly different at 5%, according to one-way ANOVA with Bonferroni* post hoc *test. Uppercase letters compare time evaluation for each resin cement (rows).*

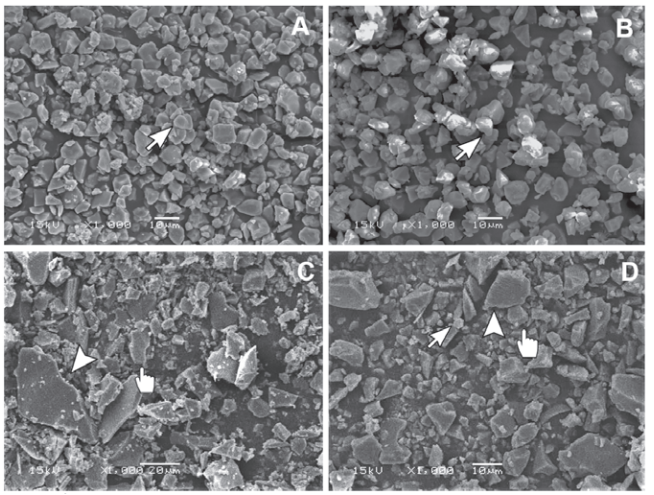

Figure 5. *SEM micrographs (1000×) of different resin cements. A, RelyX Ultimate (RXU); B, RelyX Unicem2 (RUN); C, Maxcem (MAX); D, Calibra (CAL). Legend: , spherical shape fillers with regular size;, irregular shaped particles with larger size;, irregular shaped particles with small size*

infiltration.[5,6,9] Also, the presence of acidic monomers changes the physicochemical characteristics of the resin cement and can affect the penetrability into the radicular dentin tubules, leading to failure by dislodgement of the post from inadequate adaptation.[7,8,9,27,28] Thus, a lower POBS could also be a reflection of the cement composition and the chemical reaction of each SAC tested.[26,29,30]

Another relevant factor in the analysis of the mechanical properties of resin cements is the degradation of the material.[26] This study showed that all the evaluated cements presented a decrease in the POBS after thermocycling, thus the second hypothesis, that the POBS values would not change, was rejected. This finding may be related to hydrolytic degradation of the resin matrix owing to the effect of contraction and expansion of the composite leading to adhesive failures.[31,32] Yet, when comparing the resin cements, the SACs showed lower POBS values after thermocycling than the CRCs, thus, it can be noticed that the SACs are generally more susceptible to water sorption and degradation than the CRCs.[8]

Among the evaluated SACs, MAX showed the highest POBS after 24 hours of storage with 78.91 ± 15.8 MPa and after thermocycling with 53.9 ± 11.9 MPa. These results may be related to GPDM (glycero-phosphate dimethacrylate) monomer. The GPDM is a phosphoric acid functional monomer that has two polymerizable groups, hence being more reactive and producing a higher chance to co-polymerize with other monomers, compared to the Pent-P monomer in RUN, which only contains one polymerizable group.[33] Also, GPDM has small monomers with strong hydrophilicity, inducing better dentin wettability on the hybridization process.[33] However, residual hydrophilic monomers can lead to water sorption and significant hygroscopic expansion stresses during and after the setting reaction, resulting in adhesive failures.[10,19,25,29,30]

Regarding the failure mode, adhesive failures between the cement and the dentin (type I) were the most common for all the cements even after thermocycling. This finding could be a result of an ineffective light-curing reaction.[34,35] Moazzami and others[36] showed that the apical and middle thirds of the root canals do not receive a minimal irradiance of 233 mW/cm^2, thus, photopolymerization is not adequate because of the reduced light transmission, leaving residual unreacted monomers that act as plasticizers and jeopardize the resistance of the material.[18,37]

Because the resistance of the material and the potential failure of cemented restorations are correlated to flexural strength and modulus of elasticity, these properties can be predictors of the clinical performance of resin cements. In this study, there were no significant differences on the flexural strength of the SACs RUN and CAL (94.48 and 68.40 MPa, respectively), and the CRC-RXU (91.93 MPa), although there were differences between RXU and MAX that showed a significantly lowest flexural strength of 68.40 MPa. Based on these results, the third hypothesis was partially rejected. Literature shows a high variability on the flexural strength of resin cements, reporting higher, similar, and lower values than those obtained in this study.[38,39] However, the high variability can be a result of different polymerization protocols on the specimens, and the evaluation parameters that can influence the performance and mechanical resistance of resin cements.[9,28,35]

Considering that the flexural strength and the modulus of elasticity are indicators of the ability of the material to resist deformations, resin cements should present a modulus of elasticity similar to that of dentin and the fiber post, to deform in a similar way and resist the applied loads.[5,9,12,19] MAX and CAL exhibited a low modulus of elasticity (4.91 and 4.52 GPa, respectively), while RUN showed a high modulus of elasticity (7.32 GPa) compared to RXU (6.56 GPa). The high modulus of elasticity of RUN could explain the results of failure mode, where a lower rate of type III fractures (cohesive in cement) occurred after thermocycling, which could be expected from a material with a high modulus of elasticity.

Another characteristic that influences the modulus of elasticity and flexural strength is the filler morphology.[38,39] It was observed that MAX contains mostly large filler

particles and accordingly showed a lower modulus of elasticity and flexural strength than RUN and RXU (Figure 3). The lower modulus of elasticity and flexural strength could result from a nonheterogeneous stress distribution between the particles leading to stress concentration, which produces low flexural strength. The stress concentration occurs on irregularities of the filler, angles, and protuberances; thus, the cracks initiate quickly when the filler particles are large and irregular[22] such as present in MAX (Figure 5).

Therefore, the fillers' shape and morphology could explain the highest modulus of elasticity of RUN, which contains smooth, rounded, and homogeneously sized filler particles that increased the fracture strength and improved the stress distribution. This result was followed by RXU, because both cements are developed by the same manufacturer and contain similar filler particles. Also, RUN and RXU presented a low rate of type III failure (Figure 2). This failure pattern can be a result of adequate incorporation of small particles, which allows a high number of fillers to incorporate into the resin matrix, improving the mechanical properties such as modulus of elasticity.[22] On the other hand, the presence of irregularly shaped and sized particles, as observed with CAL (Figure 5) might be preferable, because it could result in tolerance to deformation, resulting in better resistance to masticatory forces and lower fracture rates.[19,22]

It must be considered that in resin cements, filler particles such as glass may be added to regulate the acid-base reaction of the cement.[9] Thus, the ideal SAC would present a low initial pH for adequate wetting and conditioning, but, once adhesion is achieved, the pH would increase due to the acid-base polymerization reaction, leaving no residual acid monomers.[8,9,11,20] However, the results of this study confirm previous findings that report a heterogeneous pH-neutralization between cements.[10,11,19,20,30] Therefore, the fourth hypothesis was rejected, because for all the evaluated cements an initial pH decrease was observed, reaching a minimal pH after 30 minutes for MAX, and after 60 minutes for the other cements. After that, the pH increased, reaching a close to neutral (for RUN and CAL) or basic (for RXU and MAX) pH after 48 hours.

However, it must be stressed that *in vitro* evaluation of pH changes during the setting reaction has limitations, mainly because it does not consider the interactions between the cement and the tooth, hence failing to include an important element of the acid-base reaction of the cement.[8] However, it still provides relevant information to explain the POBS results. In this study, the SACs CAL and RUN had the lowest pH after 48 hours, and coincidentally also produced the lowest POBS values after 24 hours of water storage. This could be associated with the fact that after 24 hours, the pH had not been neutralized, making the cement hydrophilic and, consequently, susceptible to hydrolysis.[27,30]

Therefore, the simplification of clinical procedures by elimination of critical steps may compromise the chemical reactions and consequently the mechanical properties of the material. For SACs, the ease of use must be balanced with an adequate composition, and appropriate clinical indications. When a SAC is the preferred clinical choice, it is recommended to select a cement with a high pH neutralization, because this could mean the cement is less prone to degradation and as a result, it can be expected to exhibit better mechanical properties and stability, which are crucial for the clinical success of fiber post cementation.

CONCLUSION

It can be concluded that despite presenting a simplified technique, the mechanical behavior of SACs is not superior to that of CRCs. The CRC-RXU presented high values of POBS even after the thermocycling. Despite the limitations of this study and the variety of the composition of the resin cements, among all the SACs evaluated, MAX presented the highest POBS in 24 hours and after thermocycling evaluation. Also, it reached the closest neutral pH after 48 hours. Therefore, SACs with low initial pH and strong neutralization reactions are recommended, because these characteristics may lead to better mechanical properties and stability.

Conflict of Interest

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

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Time Frame Analysis of Potassium Nitrate and Hydrogen Peroxide Diffusion into the Pulp Chamber

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Clinical Relevance

Patient safety and satisfaction are crucial to dental practitioners. Tooth sensitivity is frequently reported as one of the most bothersome side effects of tooth-whitening. While many tooth desensitizing agents are available, there is no in-market technique that provides a desirable, time-efficient outcome.

SUMMARY

Objectives: The primary objective of this study was to evaluate the effect of an innovative double-layer, single-application desensitizing/whitening technique of potassium nitrate (PN) and hydrogen peroxide (HP) diffusion at different time points. Methods and Materials: Specimens were prepared from extracted caries-free human molars (n=90). Teeth were randomly assigned into four groups: Group A (HP CTRL) treated with 25% HP for 45 minutes, group B (PN CTRL) received a single-layer treatment of 5% PN for 45 minutes, group C received the double-layer treatment of 5% PN and 25% HP for 45 minutes, and group D received a 3% PN incorporated in a 40% HP gel for 45 minutes. PN and HP concentrations were measured at 5, 15, 30, and 45 minutes using standard chemical kits. Group comparisons were made using a repeated measures analysis of variance (ANOVA) test. Pairwise tests for differences in diffusion were done, using the Tukey adjustment of p values for multiple comparisons. A significance level of 5% was used.

Results: Group A showed no significant difference in HP diffusion rates between the 5- and 15-

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minute, 15- and 30-minute, or 30- and 45-minute time points; group D showed a similar trend; however, group C differed significantly at the 5- and 15-minute time points (p=0.0004), at the 15- and 30-minute time points (p=0.0026), and the 30- and 45-minute time points (p=0.0014). For PN diffusion, groups B and C had significantly different levels at the 15-, 30-, and 45-minute time points (p=0.0005, p=0.0002, and p<0.0001, respectively); and at the 15-, 30-, and 45-minute time points, groups D and C had significantly different PN diffusion (p=0.0327, p=0.0004, and p< 0.0001, respectively). Group C had significantly different PN diffusion at the 5- and 15-minute time points (p=0.0004), the 15- and 30-minute time points (p=0.0026), and at the 30- and 45-minute time points (p=0.0014).

Conclusion: The double-layer technique showed superior diffusion of PN into the pulp chamber and did not affect the diffusion of HP when compared to other techniques. The double-layer technique may be suggested as an alternative tooth-whitening treatment to minimize tooth sensitivity.

INTRODUCTION

Even though tooth whitening is effective, side effects still adversely affect patient compliance and satisfaction. Tooth sensitivity is the most commonly reported side effect, with an incidence rate as high as 75%.[1] The degree of sensitivity can vary from mild to intolerable, leading some patients to discontinue treatment.

A study showed that, of the subjects who underwent tooth-whitening treatment with 30% hydrogen peroxide (HP), 63% reported tooth sensitivity[2]; even higher sensitivity rates of 70% or 80% have been reported.[3,4]

Although exposed dentin, root caries, or exposed root surfaces could induce sensitivity, tooth-whitening-induced tooth sensitivity is believed to be related to the passage of HP molecules through intact dentin, dentinal tubules, and eventually the coronal part of the pulp.[5] The sensation commonly presents itself as generalized sensitivity to cold stimuli, but can also manifest as spontaneous sharp, shooting pain in a few teeth, indicating a direct effect on the nerve of the tooth.[5] HP causes cell damage or directly activates the neuronal receptor of the dental pulp complex. Recent research has shown that peroxides and other oxidizing agents oxidize cysteine residues in the TRPA1 neuron channel in the pulp complex, resulting in activation and perception of pain.[6]

Decreasing nerve excitations and blocking the transmission of pain to the central nervous system provides a potential treatment modality for tooth sensitivity. Potassium nitrate (PN) acts as a desensitizing agent, which reduces the nerve excitations by diffusing the potassium salts through enamel and dentin. These salts can reach nerve termination and affect nerve impulses. When K^+ concentration is increased above the normal physiologic level around the cell, it causes imbalance between K^+ and Na^+, and the cell depolarizes, creating a period of inactivation resulting in pain reduction or elimination.[7]

Several techniques for the delivery of PN as a desensitizing agent are available.[8] While the application of PN in a tray for 30 minutes prior to the whitening treatment[9] succeeded in reducing tooth sensitivity, this two-step technique was time consuming and inconvenient for the patient and clinician. Incorporating PN in the whitening gel[10] was introduced, eliminating the two-step application protocol, but similar diffusion times into the pulp chamber for both PN and HP were reported. Studies have shown that PN could be detected as early as five minutes[11] and HP as early as five to 10 minutes.[12] We believe this mixed approach is not effective due to the similar diffusion time of both PN and HP and, because of the lower molecular weight of HP, we assume that hydrogen will diffuse faster through the tooth and activate neuronal receptors in the pulp complex; by that time, reducing transmission of nerve impulses by PN is no longer possible.

To overcome these drawbacks, an innovative new technique, "the double-layer technique,"[13] utilizes a layer of PN covered by a layer of HP. This technique combines efficiency and efficacy, and will result in a better diffusion of PN. Since the PN layer will act as a physical barrier to the HP layer, we believe that PN will have a head start on diffusion. Hence, the purpose of this study is to establish a timeframe in which PN and HP diffusion are detected within the pulp chamber and to also evaluate the effect of PN on HP diffusion.

Our hypotheses were: 1) There is no difference in HP and PN diffusion times into the pulp chamber when used in different tooth-whitening techniques, and 2) HP and PN are not detected within the pulp chamber at, and at time points after, five minutes.

METHODS AND MATERIALS

Sample Preparation

Recently extracted human molars without any identifiers were obtained from the Iowa Institute for Oral Health Research at the University of Iowa. Teeth were thoroughly cleaned using non-fluoridated pumice

(Pumice Preppies, Whip Mix, Louisville, KY, USA) and prophy cups (Young Dental, Earth City, MO, USA) to remove debris from the tooth surface that was treated. Teeth were examined for caries, restorations, fractures. and cracks; teeth with these conditions were excluded. Teeth were sectioned 3 mm apical to cementoenamel junction (CEJ): 1) to achieve an open pulp chamber, and 2) to enlarge the pulp chamber vertically to be able to retain the 100-μL buffer solution that acted as a reservoir for the nitrate/HP residue. Diamond burs were used to enlarge the pulp chamber circumferentially to allow for the retention of assay solutions.

Following chamber enlargement, the remaining tooth thickness was measured using a boley gauge (HuFriedy Group, Chicago, IL) to ensure that a 2-mm wall thickness remained in all specimens. The average thickness is 2 mm, and for standardization purposes, all samples were reduced to a thickness of 2 mm.

A standardized circular treatment area 6 mm in diameter was established on the buccal surface. This aided in standardizing the treatment application along with treatment gel thickness.

Treatment and Group Distribution

Treatment application time and gel concentration followed the manufacturers' guidelines to resemble a clinical setting. Group A (HP control) was treated with 25% HP for 45 minutes (Philips Zoom WhiteSpeed, Philips Oral Healthcare, Stamford, CT, USA); group B (PN control) received a single-layer treatment of 5% PN (Relief ACP, Philips Oral Healthcare, Stamford, CT, USA) to act as a baseline for the amount of PN diffusion for 45 minutes; group C received the double-layer treatment (Figure 1) of 5% PN and 25% HP for 45 minutes; and group D received 3% PN incorporated in a 40% HP gel (Opalescence Boost PF, Ultradent, South Jordan, UT, USA) for 45 minutes. The amount of PN and HP in the pulp chamber was measured using standard chemical assays.

A sealed tooth-whitening technique was used for the groups using HP; HP was covered with a linear, low-density polyethylene wrap (Professional Plastic Food Wrap Film, Bakers & Chefs, Bentonville, AR, USA) to avoid evaporation. Since HP needs to be replenished every 15 minutes, this technique eliminated the need to replenish the gel during the tooth-whitening procedure for 45 minutes.[14] Groups treated with Zoom HP were continuously exposed to a light-emitting diode (LED: Zoom WhiteSpeed, Philips Oral Healthcare, peak wavelength: 466 nm) set at high intensity (190 mW/cm^2) throughout treatment time.

Gel quantity was measured using the formula $V=\pi r^2 h$. For PN, measurements were as follows: r=3 mm, h=1 mm, V=28.27 mm^3=28.27 μL. Values for HP were: r=3 mm, h=2 mm, and V=56.54 mm^3=56.54 μL.

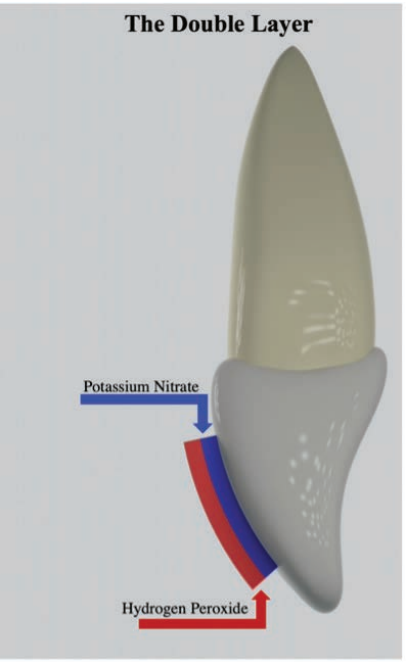

Figure 1. *Illustration of the application of the double layer technique*

Time Point Measurements

For quantifying the concentration of HP and PN within the pulp chamber at different time points, HP and/or PN residue within the pulp chamber was measured at 5, 15, 30, and 45 minutes in all groups. Choosing these time points was based on the amount of time PN and HP had been applied clinically. At these time points, a sample was withdrawn from the tooth's enlarged pulp chamber, and the amount of HP or PN residue within the pulp chamber was measured.

Hydrogen Peroxide Calculation

Acetate buffer solution was placed in the pulp chamber to calculate the amount of HP residue. Using a Fluorimetric HP assay kit and following the manufacturer's instructions (Sigma-Aldrich Co LLC, St Louis, MO, USA). A master mix was formulated by combining 50 μL Red Peroxidase Substrate Stock, 200 μL Horseradish Peroxidase, and 4.75 mL Assay Buffer. To calculate HP concentration within the sample, 50 μL of the Master Mix was added to each of the sample wells (50 μL sample volume), and the plate was incubated at room temperature for 15 to 30 minutes. The intensity of the color was proportional to the HP concentration and was measured in a UV/visible spectrophotometer at a wavelength of 596 nm

against a reference prepared in the same manner but without HP using a microplate reader.

Potassium Nitrate Calculation

One hundred microliters of phosphate buffer solution were placed in the pulp chamber. This buffer solution acts as a reservoir for the PN that possibly diffuses through the tooth into the pulp chamber after treatment. A colorimetric nitrate/nitrite assay kit (Sigma-Aldrich Co LLC) composed of nitrate and nitrite standard solutions, buffer solution, nitrate reductase, enzyme co-factors, and Griess dyes were used for the colorimetric determination of nitrate in the samples. The nitrate and nitrite concentration in the sample solution was calculated. Further, the final nitrate concentration in the sample solution was obtained by the following equation[15]: [Nitrate]=[Nitrate/Nitrite]-[Nitrite].

Statistical Analysis

Based on pilot data available to help guide the sample size determination, 15 samples/group were deemed sufficient to detect the desirable difference between groups. Diffusion of HP and PN was repeatedly measured at 5, 15, 30, and 45 minutes using donated extracted teeth, n = 15 in each group (n=90 total). The concentration was measured separately for four preparation combinations of HP and PN, resulting in six groups to compare in total. The groups were defined as the following:

- Group A: 25% Hydrogen Peroxide-A-HP (n=15)
- Group B: 5% Potassium Nitrate-B-PN (n=15)
- Group C: 5% PN, 25% HP-C-PN (n=15) & C-HP (n=15)
- Group D: 3% PN, 40% HP-D-PN (n=15) & D-HP(n=15)

Two repeated measures of analysis of variance (ANOVA) were done; both analyses assumed a compound symmetric correlation matrix. Tests of overall significance were conducted to determine if there is an overall effect of time, group, and the interaction of group and time. Following this, pairwise tests for difference in diffusion were done, using the Tukey adjustment of p values for multiple comparisons.

RESULTS

Hydrogen Peroxide

Summary statistics for the HP groups are found in Table 1 and Figure 2.

For group A-HP there was not a significant increase in concentration between consecutive time points; although the A-HP concentration did increase between the beginning and later time points (5 to 45 minutes, $p<0.0001$). Similarly, for group D, there was no significant difference between any consecutive time points. However, there was a significant difference between time points 5 and 45 minutes ($p=0.0025$). Group C showed a different trend compared to groups A-HP and D-HP. HP concentration for group C was significantly different at time points 5 and 15 minutes ($p=0.0004$), at time points 15 and 30 minutes ($p=0.0026$), and time points 30 and 45 minutes ($p=0.0014$). See Table 2.

The differences between groups at each time point can also be considered. At time points 5, 15, and 30 minutes,

Table 1: *Summary Statistics for the Concentration of Hydrogen Peroxide by each Time Interval*

Group	Time (Min)	Mean (μM)	SD (μM)	Median (μM)
A-HP	5	57.66	47.91	25.05
C-HP	5	33.96	21.35	28.5
D-HP	5	60.6	44.26	33.57
A-HP	15	103.13	55.5	82.88
C-HP	15	144.18	99.98	145.87
D-HP	15	107.82	59.13	111.73
A-HP	30	170.77	76.36	175.41
C-HP	30	243.46	166.43	254.52
D-HP	30	135.37	79.06	125.68
A-HP	45	236.96	88.51	234.66
C-HP	45	346.84	225.68	370.8
D-HP	45	160.08	96.67	130.45

Abbreviations: A-HP, 25% HP; C-HP, 5% PN, 25% HP; D-HP, 3% PN, 40% HP; HP, hydrogen peroxide; PN, potassium nitrate.

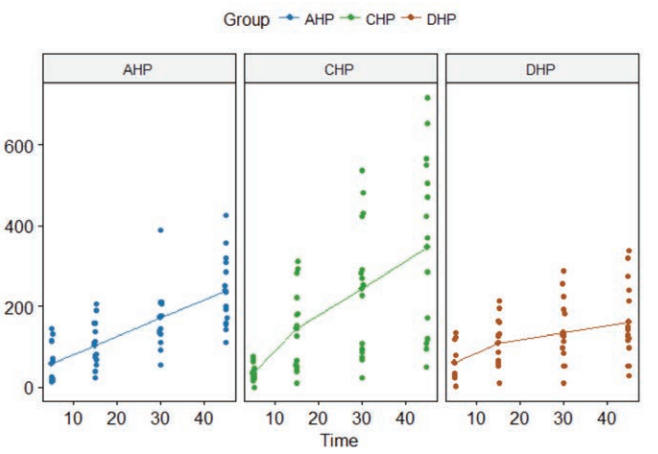

Figure 2. *Plot of hydrogen peroxide concentration (μm) with time (minutes), by group with means*

Table 2: *Repeated Measures ANOVA Results for Hydrogen Peroxide* (p-*value*) *per Group*

Group 1	Time 1 (Min)	Group 2	Time 2 (Min.)	Adj *p*-value
A-HP	5	A-HP	15	>0.05
A-HP	15	A-HP	30	>0.05
A-HP	30	A-HP	45	>0.05
C-HP	5	C-HP	15	0.0004
C-HP	15	C-HP	30	0.0026
C-HP	30	C-HP	45	0.0012
D-HP	5	D-HP	15	>0.05
D-HP	15	D-HP	30	>0.05
D-HP	30	D-HP	45	>0.05
A-HP	5	C-HP	5	>0.05
C-HP	5	D-HP	5	>0.05
A-HP	15	C-HP	15	>0.05
C-HP	15	D-HP	15	>0.05
A-HP	30	C-HP	30	>0.05
C-HP	30	D-HP	30	>0.05
A-HP	45	C-HP	45	>0.05
C-HP	45	D-HP	45	0.0002

Abbreviations: A-HP, 25% HP; C-HP, 5% PN, 25% HP; D-HP, 3% PN, 40% HP; HP, hydrogen peroxide; PN, potassium nitrate.

there were no significant differences in concentration among the three HP groups. At the 45-minute time point, C-HP and D-HP were significantly different (p=0.0002).

Potassium Nitrate

Summary statistics for the PN groups are found in Table 3 and Figure 3.

For group B-PN, there were no significant differences between the 5- and 15-minute time points or between the 30- and 45-minute time point; however, there was a significant difference between the 15- and 30-minute time points (p=0.0137). For group D, there were no differences between any consecutive time points; however, there were differences between the early and later time points (Table 4). For group C, there was a difference between every consecutive time point (5- vs 15-minutes, p=0.0003; 15- vs 30-minutes, p=0.0034; and 30- vs 45-minutes, p=0.0002). So, groups B and D had little diffusion of PN over time, but group C showed a consistent increase.

At the 5-minute time point, there were no differences among any of the groups. At the 15-, 30-, and 45-minute time points, groups B and C had significantly different PN concentrations (p=0.0005, p=0.0002, and p<0.0001, respectively). Also, at the 15-, 30-, and 45-minute time points, groups C and D had significantly different PN concentrations (p=0.0327, p=0.0004, and p<0.0001, respectively). However, groups B and D did not show significantly different concentrations of PN at any time point (Table 4).

Table 3: Summary Statistics for the Diffusion of Potassium Nitrate by Time

Time (Min)	Group	Mean (µM)	SD (µM)	Median (µM)
5	B-PN	0.83	3	0
5	C-PN	8.66	7	11.49
5	D-PN	3.07	7.84	0
15	B-PN	1.52	5.49	0
15	C-PN	22.82	4.97	22.18
15	D-PN	7.29	11.22	0
30	B-PN	13.04	14.86	10.99
30	C-PN	35.12	7.08	35.35
30	D-PN	14.02	14.46	13.24
45	B-PN	20	21.51	16.09
45	C-PN	49.42	16.16	47.57
45	D-PN	18.84	13.42	17.84

Abbreviations: B-PN, 5% potassium nitrate; C-PN, 5% PN, 25% HP; D-PN, 3% PN, 40% HP; PN, potassium nitrate.

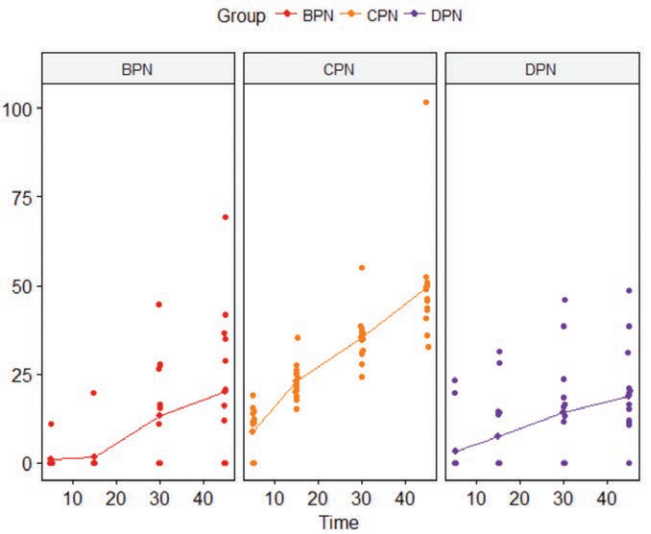

Figure 3. *Plot of potassium nitrate concentration (µm) with time (minutes), by group with means*

Table 4: *Repeated Measures ANOVA Results for Potassium Nitrate (*p*-value) by Group*

Group 1	Time 1 (Min)	Group 2	Time 2 (Min)	Adj *p*-value
B-PN	5	B-PN	15	>0.05
B-PN	15	B-PN	30	<0.013
B-PN	30	B-PN	45	>0.05
C-PN	5	C-PN	15	<0.0003
C-PN	15	C-PN	30	<0.003
C-PN	30	C-PN	45	<0.0002
D-PN	5	D-PN	15	>0.05
D-PN	15	D-PN	30	>0.05
D-PN	30	D-PN	45	>0.05
B-PN	5	C-PN	5	>0.05
C-PN	5	D-PN	5	>0.05
B-PN	15	C-PN	15	0.0005
C-PN	15	D-PN	15	0.0327
B-PN	30	C-PN	30	0.0002
C-PN	30	D-PN	30	0.0004
B-PN	45	C-PN	45	<0.0001
C-PN	45	D-PN	45	<0.0001

Abbreviations: B-PN, 5% PN; C-PN, 5% PN, 25% HP; D-PN, 3% PN, 40% HP; PN, potassium nitrate.

DISCUSSION

Tooth whitening is the first approach when considering enhancing tooth color. The at-home approach is the most popular due to its low cost and minimal chair time. However, people feel safe under a dentist's supervision, thus the in-office approach is emerging as the most desirable with less time to reach results. Using high concentration HP presents issues such as high prevalence of tooth sensitivity.[16] The use of desensitizing agents has proved to be beneficial to overcome this unwanted side effect.[8] However, using the desensitizing agent in a tray prior to tooth whitening is time consuming, and studies have shown lower efficacy in combining the desensitizing agent with the tooth whitening agent.[17] The double-layer technique reduced the desensitizing/tooth whitening protocol and presents an approach that favors the diffusion of the desensitizing agent.

The purpose of this study was to establish a time frame at which PN and HP concentrations are detected within the pulp chamber and also evaluate the effect of PN and HP diffusion. It is crucial for PN to be detected at early stages in the tooth-whitening treatment to be effective. In this study, different modalities of the application of PN were evaluated to understand its mechanism of diffusion.

Diffusion is the movement or spreading of molecules from a high concentration to a low concentration area. The molecular weight of a compound is crucial, with the lower molecular weight compounds generally diffusing faster. The molecular weight of PN is 101.1 g/mol compared to HP, which is 34.01 g/mol. Diffusion is influenced by factors such as pressure and temperature, in which more pressure or higher temperature increases the rate of diffusion.[18,19] In dentistry, the concentration of the agent, thickness of the tooth, and diameter of the dentinal tubules are additional factors to be considered.[11,20]

In this study, we evaluated different hypotheses regarding the diffusion of HP and PN. Our hypothesis was that HP diffusion is similar regardless of the method used. Our results support the rejection of the first null hypothesis. When considering the different time points (5, 15, 30, and 45 minutes), group A-HP and D-HP showed no significant difference between time points. However, group C-HP showed a different trend. The HP diffusion for group C-HP was significantly different at consecutive time points. So, while A-HP and D-HP showed modest increases in diffusion over time, C-HP showed a consistent increase in diffusion. The increase of HP diffusion over time in this study is in agreement with other studies that found that the diffusion of HP is time dependent.[21,22]

For diffusion, there was no statistically significant difference between group C-HP at 30 minutes and group A-HP or D-HP at 45 minutes (p>0.05 for both comparisons). Our innovative tooth-whitening technique showed equivalent diffusion of HP with less treatment time. Studies have reported that HP diffusion is directly correlated with tooth color change,[20,23] and we believe that with our technique, treatment time could be reduced and tooth color change could be reached more efficiently when compared to other techniques.

Concentration of HP is another factor that influences diffusion, since higher concentration creates a larger driving force for diffusion[11,24]; in this study, we compared concentrations of 25% and 40% HP. At the end of treatment, data showed a significant increase in diffusion with 25% when compared to 40% HP (p=0.0002). In this study, diffusion was not concentration-dependent, thus we believe that other factors such as temperature could have substantial impact on diffusion.[20,25] LED light was used with 25% HP so that, through thermocatalysis, light energy was converted to heat to increase efficiency of the tooth-whitening agent.[26]

Our hypothesis stated that HP is not detected in the pulp chamber at 5, 15, 30, and 45 minutes, and our data support the rejection of the hypothesis as concentrations of HP were found at all time points. Our results are in agreement with other studies that showed HP could be detected in the pulp chamber as early as 5 minutes.[22,27] Tooth-whitening sensitivity is believed to be related to the diffusion of HP. Fast diffusion of HP posed a dilemma for what is the best approach to overcome this issue; PN regimens were effective but did not fully eliminate tooth sensitivity. PN needs to be detected in pulp chamber early to mask the side effect of HP.

Considering PN diffusion, our hypothesis stated that there is no difference in PN diffusion into the pulp chamber when used in different tooth-whitening techniques. In this study PN was used alone, mixed into the tooth-whitening agent, or placed as a first layer in the double-layer technique. Our results support the rejection of the hypothesis: at the 15-, 30-, and 45-minute time points, groups B-PN and C-PN had significantly different PN diffusion (p=0.0005, p=0.0002, and p<0.0001, respectively), and at the 15-, 30-, and 45-minute time points, groups D-PN and C-PN had significantly different PN diffusion (p=0.0327, p=0.0004, and p<0.0001, respectively). Although PN diffusion increased at each time point, groups B-PN and D-PN showed no significant difference between any consecutive time points. However, group C-PN showed a different trend compared to groups B-PN and D-PN: PN was significantly different at the 5- and 10-minute time points (p=0.0004), the 15- and 30-minute time points (p=0.0026), and the 30- and 45-minute time points (p=0.0014). Our data are in agreement with studies that proved that PN is time dependent.[14]

The double-layer technique showed superior diffusion when compared to other techniques. At 5 minutes, the mean PN residue in the pulp chamber was 0.83 μM, 8.66 μM, and 3.07 μM for groups B-PN, C-PN, and D-PN, respectively; having higher PN diffusion is indicative of surplus potassium salts that interact with the nerve endings and could cause a prolonged desensitizing effect (Table 3). In this study, a number above 10 μM is of interest, as a study by Hodgkin and others[28] looked at the amount of PN diffusion when effective desensitization happens. In that study, they concluded that an increase of K^+ in the vicinity of the interdental nerve endings by 10 μM inhibits nerve impulses. When we look at our results reported, and with the limitation of this being an *in vitro* study, we can say an effective desensitization happens in group B-PN and D-PN around 30 minutes, while desensitization will happen earlier in group C-PN, between 10 to 15 minutes.

Faster diffusion was observed when HP was included, regardless of the technique; the viscosity of the compound might have contributed to the reported results as studies have concluded that viscosity is correlated with diffusion.[14,29] The use of light as a supplement to the tooth-whitening procedure was investigated in many studies. Light produces heat and whenever heat is exerted on the tooth-whitening agent, the temperature of the agent increases and increases its efficiency in releasing hydroxyl free radicals.[26,30] Superior diffusion of PN was observed in the double-layer technique when compared to PN control and the mixed technique as LED light was used in this technique, which could have an influence on the PN diffusion. However, there are no studies on the effect of light on a desensitizing agent's diffusion.

This *in vitro* study is one of few studies that evaluated the diffusion of PN into the pulp chamber and added to the literature regarding HP diffusion. The results of our study are somewhat challenging to compare to an *in vivo* setting. *In vivo* studies have two added variables-positive pulpal pressure and osmotic pressure of the gels[31]-that significantly affect diffusion.

Tooth preparation to encompass the 100 μL of buffer was another limitation since it is not a representation of a clinical situation. However, the tooth preparation protocol in our study took into consideration the safe amount of buccal thickness to be left, closely mirroring *in vivo* settings. The diffusion of PN into the pulp chamber has been investigated only recently. The limited literature on this topic showed that diffusion of PN into the pulp is controlled by different factors, such as concentration, viscosity, and other internal factors. It is crucial for the management of tooth-whitening-induced sensitivity that future studies should be directed toward innovative techniques that are effective and easy to implement.

A double layer, PN covered by HP, was evaluated to assess whether this technique will have an adverse effect on the diffusion of HP. Findings from our study showed promise in the advancement of managing tooth sensitivity. A model could be suggested in which a double layer of PN and HP could be formulated with the addition of a sealing varnish that provides isolation from the outer environment.

An *in vivo* study should be conducted to evaluate this technique, assess its effectiveness in managing tooth sensitivity, and analyze its effect on tooth color change, with a consideration of the clinical variables that might influence the outcome of such a technique.

CONCLUSIONS

This study evaluated whether the application of PN as a desensitizing agent prior to in-office tooth whitening

impairs HP diffusion. It also evaluated whether an in-office whitening agent impairs the diffusion of PN.

The findings of this study showed that the application of PN as a layer prior to an in-office tooth-whitening agent does not impair HP diffusion. Additionally, the application of the in-office whitening agent did not impair the diffusion of PN into the pulp chamber.

Within the limitations of this study, the double-layer technique showed superior diffusion of PN into the pulp chamber when compared to other techniques. The double-layer technique increased the efficiency of PN diffusion. The double-layer technique may be suggested as an alternative tooth-whitening treatment to minimize tooth sensitivity.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of the University of Iowa. The approval code issued for this study is 201710755.

Conflict of Interest

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

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Influence of Viscosity and Thickener on the Effects of Bleaching Gels

CRG Torres • SE Moecke • APVP Mafetano • LF Cornélio • R Di Nicoló • AB Borges

The viscosity and kind of thickener can have a significant influence on the efficacy and safety of tooth whitening treatment and is as important to the development of optimized gel formulations as the hydrogen peroxide active ingredient.

http://doi.org/10.2341/20-309-L

Can Specular Gloss Measurements Predict the Effectiveness of Finishing/Polishing Protocols in Dental Polymers? A Systematic Review and Linear Mixedeffects Prediction Model

TP de Melo • AHS Delgado • R Martins • L Lassila • S Garoushi • J Caldeira • AM Azul • P Vallittu

A clear and dependent relationship was found between specular gloss and roughness in resin composites. A reference value of >55 GU was found to be correlated with well-polished samples. This value can thus be used to objectively determine effectiveness of polishing and may serve as a starting point for future in vivo gloss measurements.

http://doi.org/10.2341/21-027-LIT

Development and Assessment of Bioactive Coatings for the Prevention of Recurrent Caries Around Resin Composite Restorations

LM Firoozmand • Y Alania • AK Bedran-Russo

The application of bioactive surface coatings potentially contributes to the in vitro prevention of recurrent caries in enamel and dentin—a major cause of failure of resin composite restorations.

http://doi.org/10.2341/20-299-L

Use of Computerized Microtomography, Energy Dispersive Spectroscopy, Scanning Electron Microscopy, and Atomic Force Microscopy to Monitor Effects of Adding Calcium to Bleaching Gels

LC Mendonça • MLA Rodrigues • AA Bicalho • GR daSilva • PS Quagliatto • CJ Soares

Bleaching teeth with hydrogen peroxide gels containing calcium does not prevent mineral loss at the enamel surface. However, the demineralized regions do not exhibit an increase in surface roughness.

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Influence of Viscosity and Thickener on the Effects of Bleaching Gels

CRG Torres • SE Moecke • APVP Mafetano • LF Cornélio • R Di Nicoló • AB Borges

Clinical Relevance

The viscosity and kind of thickener can have a significant influence on the efficacy and safety of tooth whitening treatment and is as important to the development of optimized gel formulations as the hydrogen peroxide active ingredient.

SUMMARY

Objective: This study investigated the influence of the viscosity and kind of thickener of 35% hydrogen peroxide bleaching gels on the tooth (color change, demineralization of enamel, and permeation) and on the gel [reactive oxygen species (ROS), pH, and peroxide concentration].

Methods and Materials: Two hundred forty specimens were divided into groups of bleaching gels with different thickeners (CAR, carbomer; ASE, alkali swellable emulsion; MSA, modified sulfonic acid polymer; SSP, semisynthetic polysaccharide; PAC, particulate colloids) in three viscosities (low: 50,000 cP; medium: 250,000 cP; high: 1,000,000 cP). Color change (Δ*E*ab), demineralization of enamel by Knoop microhardness (KHN) reduction analysis, and peroxide permeation (PP) were analyzed in the specimens, while pH, peroxide concentration (PC), and ROS were evaluated in the gels. Data were analyzed by two-way ANOVA (α=0.05).

Results: The higher viscosity gels reduced Δ*E*ab, PP, enamel softening, and ROS in relation to the lower viscosity gels. However, the drop in pH and PC were higher in the more viscous gels. Gels with MSA produced higher Δ*E*ab compared with SSP and ASE. The PP was higher for PAC, and smaller for SSP and CAR. The KHN reduction was higher for CAR and smaller for PAC. The higher pH reduction was seen for ASE and CAR, and

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the smaller for SSP. The PC reduction was higher for SSP and smaller for CAR. More ROS were observed for MSA and fewer for ASE.

Conclusions: Increased gel viscosity was associated with reduced color change, permeation, demineralization of enamel, and ROS, and led to increased peroxide decomposition and pH alteration during the treatment. The kind of thickener significantly interfered with the treatment effects.

INTRODUCTION

Tooth color is a result of the penetration into the enamel and dentin of light, which is scattered, partially absorbed, and reflected to observers. The unabsorbed wavelengths leave the tissue creating the color sensation; this depends on the interaction of light with certain molecules inside the mineralized tissues, which act as pigments or dyes.[1] Parts of these molecules (atom or group of atoms), known as chromophores, are responsible for its light absorption.[2] The small molecules of hydrogen peroxide, the most commonly used bleaching agent in dentistry, easily penetrate the tooth structure. Because of the inherent instability of hydrogen peroxide, it undergoes O-O bond homolysis, creating reactive oxygen species (ROS), also known as free radicals.[2,3] The ROS promote the oxidation of chromophore groups in the colored molecules inside enamel and dentin, reducing light absorption, and producing a chromatic change and whitening effect.[1,4–6]

Bleaching treatment can be performed at home by using whitening agents with low peroxide concentrations for longer periods or in the dental office by using higher concentrations for shorter periods.[6] Regardless of the technique and peroxide concentration, to allow a controlled application onto the tooth structure, the bleaching agent must contain a thickening agent, creating an appropriate gel consistency and allowing for topical application.[7] A gel is a semisolid, containing small, colloidal inorganic particles or organic molecules (thickeners) dispersed in an interpenetrated liquid.[8] These particles may attract and bond to neighboring ones, creating a three-dimensional network that immobilizes the liquid, in this case, the hydrogen peroxide and water, preventing flow when in a steady-state and increasing the viscosity of the system.[9] Viscosity is a physical property of fluids related to their resistance to deformation because of internal frictional forces when in relative motion. The amount of thickener and the strength of the molecular interaction will determine the viscosity of the product.

The gel is a semisolid "dosage form" used to facilitate the administration and delivery of a medicament (active ingredient or drug) to a patient for different applications,[8] such as skin care, dermatology, gynecology, and oral care. Internal diffusivity of the active ingredient inside the product has been reported to be inversely proportional to the viscosity of the medium[10,11] and directly proportional to the size of the penetrant molecule, as predicted with the Stokes–Einstein equation.[10,12] The composition of the thickener and its different electrical charge has been reported to result in different diffusivity of the drug, even in similar concentrations, because of the interaction between the electrical charge of the thickener and the drug.[12] Additionally, in different areas of medicine, studies have reported the negative impact of the higher viscosity of semisolid vehicles caused by the increase of thickener concentration on the drug release and the permeation into the tissues.[10,13–20] In addition, because of large differences among the thickener molecules, each specific agent produces a different kind of three-dimensional network responsible for the gel formation, which also can interfere with drug delivery. Therefore, to optimize formulation, the physicochemical factors that influence the release properties must be understood. Different kinds of thickener, as well as viscosity, may affect the interaction of peroxide-based bleaching gels with the tooth structure.

Once the peroxide has been delivered by the gel, it penetrates the intercrystalline spaces of the enamel and into the dentin tubules, reaching the pulp chamber. On its way, it interacts with tooth chromophores, creating the bleaching effect, and also with organic and inorganic substances of the dental tissues, producing side effects that should be minimized. The first is the demineralization of enamel, caused by the undersaturation of bleaching gels with respect to Ca^{2+} and PO_4^{3-} ions,[21] low pH,[22] or oxidation of enamel proteins.[23] In addition, some anionic thickeners have been reported to promote the demineralization of enamel by chelation,[24] reducing microhardness, and possibly increasing tooth wear[25] and staining.[26] When they reach the pulp, the ROS produce cell damage, interfering with the cellular metabolism and vascular permeability, resulting in an inflammatory response at various levels[27] and pain.[28] Therefore, to improve bleaching therapy, the aim is to reduce the side effects while maintaining or improving the whitening efficacy.

During the bleaching procedure, the pH of gels applied over the tooth surface reduces at a variable rate according to the specific formulation.[29] This occurs simultaneously with a reduction in peroxide concentration[30] because of decomposition and

permeation toward the tooth. However, the number of free radicals inside the peroxide solution is directly proportional to the pH,[31] and it has been reported that bleaching efficacy is associated with a higher pH.[32,33] Therefore, pH and peroxide concentration should be kept at a stable level while applied to the tooth to reduce the demineralization of enamel and maintain the whitening effect. The kind of the thickener and the level of viscosity might affect peroxide penetration and the presence of ROS in the pulp chamber.

Although extensively studied in other medical specialties,[10,34–38] the authors are aware of only one study that evaluated the effect of the vehicle's viscosity on the bleaching efficacy.[39] Products from each manufacturer have different formulations and contain different excipients in different amounts, which results in quite different rheological properties. Knowledge of the effect of viscosity on a product's efficacy is typically proprietary, and under the control of the dental company's research and development department. That the full composition of each product is generally not released hampers an intensive and specific analysis of the topic.

Considering the paucity of scientific information available, the aim of this study was to investigate the influence of the viscosity and kind of thickener in 35% hydrogen peroxide bleaching gels on the tooth structure (color change, demineralization of enamel, and permeation) and inside the gel (ROS, pH, and peroxide concentration) during the application. The null hypotheses tested were that the viscosity and the kind of thickener in the formulation would not interfere with the effect of the bleaching treatment and the reactions inside the gel.

METHODS AND MATERIALS

Sample Size Calculation

Considering a statistical power of 80%, α error probability of 5%, effect size of 30%, and 15 experimental groups, the sample size was calculated for ANOVA using the G*Power 3.1.9.7 software (Franz Faul, Universität Kiel, Germany), and the minimum total sample size was 216 specimens.

Specimen Preparation

Two hundred forty cylindrical specimens (6 mm in diameter) were obtained from the labial surface of bovine incisors with a diamond trephine mill in a circular cutting machine under water cooling (Figure 1A). Enamel and dentin surfaces were flattened to create a disk shape, and the final thickness standardized at 2 mm (1 mm of enamel and 1 mm of dentin) (Figure 1B). The enamel surface was polished by using an automatic polishing machine (Panambra, Sao Bernardo do Campo, Brazil) and SiC abrasive paper grits P1200, P2400, and P4000 (Extec Corp, Enfield, USA) for 30, 60, and 120 s, respectively.[40] The disks were ultrasonically cleaned after the use of each abrasive paper to remove residue. The dentin side of the

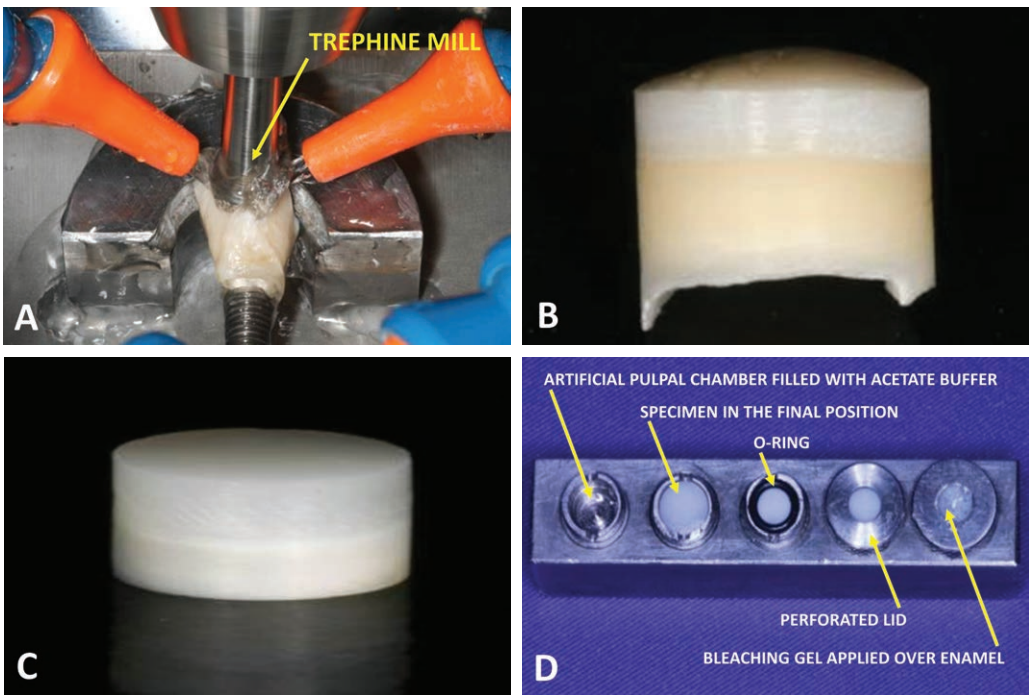

Figure 1. *Sample preparation and experimental set-up. (A) Specimen sectioning from the buccal surface of bovine incisor. (B) Specimen removed from the tooth. (C) Standardization of enamel and dentin thickness. (D) Different steps for assembling the enamel/dentin disk into the artificial pulp chamber.*

specimens was etched for 15 s using a 35% phosphoric acid gel (Ultra-Etch, Ultradent Products, Inc, South Jordan, UT) to remove the smear layer created by the grinding procedure, opening the dentin tubules to simulate the clinical condition.[41,42] The specimens were then immersed in individual Eppendorf tubes containing 2 mm of ultrapure water for rehydration. Each tube was numbered to identify the specimen.

Formulation of Gels

Experimental bleaching gels were prepared using five different thickeners, as shown in Table 1. Each thickener was combined with 60% hydrogen peroxide solution (Kolovec, Diadema, SP, Brazil), ultrapure water, and pure triethanolamine (TEA) to adjust the pH, creating 35% hydrogen peroxide gels (w/w) with a pH of 6.5. The amount of TEA used in each formulation (w/w) for low, medium, and high viscosity, respectively, for the different thickeners were as follows: Carbopol (2.92, 3.53, 3.93), Salcare (3.66, 4.86, 7.96), Aristoflex (0.50, 0.62, 0.87), Aerosil (1.33, 0.90, 0.36), and CMC (0.59, 4.86, 7.04). For the semisynthetic polysaccharide (SSP) thickener, a small amount of propylene glycol was used to prehydrate the powder, and to allow water hydration and gel formation.[9] The gels were mixed with a dual asymmetric centrifugal laboratory mixer (model DAC 150.1 FVZ, SpeedMixer, FlackTek, Landrum, USA).

The amount of each thickener was adjusted to obtain different levels of viscosity and measured with a Brookfield DV2T Viscometer (Brookfield Engineering Laboratories, Middleboro, MA, USA), as previously described,[14–19,35,36,43] and associated with the Helipath stand and a T-Bar Spindle (#96),[44] inside a 10-mL Becker flask at 25°C. Three different viscosities were created, named low (50,000 cP), medium (250,000 cP), and high (1,000,000 cP).

Therefore, 15 experimental gels were created from the combination of the five thickeners (CAR, MSA, ASE, SSP, and PAC) and three viscosities (low, medium, and high). In addition, a negative control group (NC) was treated with ultrapure water adjusted to pH 6.5 with TEA.

The baseline hydrogen peroxide concentration (w/w) in each bleaching gel was checked by titration with potassium permanganate by using a potentiometric titrator (HI902C1-02, Hanna Instruments, Woonsocket, USA) with an ORP electrode (HI3618D, Hanna Instruments).[30] The pH was measured with a pH meter (Digimed, Sao Paulo, Brazil) and a microbulb electrode (HI1083B, Hanna Instruments). The ROS quantification in the bleaching gel was performed with aminophenyl fluorescein (A36003, Invitrogen, Paisley, UK), which has a high fluorescence response to the hydroxyl free radical (•OH). The fluorescence was measured by wavelength excitation and detection of 485 nm/528 nm, respectively, with a multimode microplate reader (Synergy HTX, Biotek, Vermont, USA). The results were obtained in relative fluorescent units (RFU).

Baseline Measurements of the Specimens

Color measurements were made with a colorimetric spectrophotometer (CM 5, Konica Minolta, Osaka, Japan) and adjusted for small area view (SAV) with an aperture size of 3 mm, D65 standard illuminant, and 2° standard observer and specular component included (SCI). The specimens were removed from the water, gently dried with an absorbent paper to avoid

Table 1: *Information about the Thickeners Tested*

Thickener		Substance Name	Trademark	Charge	MW	CAS	Manufacturer
CAR	Carbomer (CAR)	Homopolymer of acrylic acid	Carbopol 980	Anionic	104,400	9003-01-4	Lubrizol (Wickliffe, Ohio, USA)
MSA	Modified sulfonic acid (MSA) polymer	Ammonium acryloyl dimethyl taurate / vp copolymer	Aristoflex AVC	Anionic	>10,000	335383-60-3	Clariant (Muttenz, Switzerland)
ASE	Alkali swellable emulsion (ASE)	Ethyl acrylate, n-butyl acrylate, methacrylic acid polymer	Salcare SC 81	Anionic	314.37	9033-79-8	BASF (Ludwigshafen, Germany)
SSP	Semi-synthetic polysaccharide	Sodium carboxymethylcellulose (CMC)	Denvercel	Anionic	262.19	9004-32-4	Denver (Cotia, SP, Brazil)
PAC	Particulate colloids (PAC)	Silicon dioxide (pyrogenic silica)	Aerosil 200	Non-ionic	60,084	7631-86-9	Evonik (Essen, Germany)

Abbreviations: MW, molecular weight (g/mol).

dehydration, and placed into the specimen's reading holder. A standard white background was placed on the dentin side.[40,45] The area was covered with a completely opaque black color cylinder (Zero Calibration Box, CM-A124, Konica Minolta), preventing any interference of environmental light on the color reading. The reflectance data of each specimen was converted to the chromatic coordinates *L**, *a**, and *b** by using the SpectraMagic NX software program (Konica Minolta).

Enamel surface microhardness was measured with a Knoop indenter coupled to a microhardness tester (FM-700, FutureTeck, Tokyo, Japan) using a load of 50 g and a 10-second dwell time. Three indentations were performed 100 μm apart, and the mean Knoop hardness number (KHN) for each specimen was calculated. Considering the baseline microhardness values, the specimens were stratified into 16 groups (n=15), corresponding to 15 experimental gels and one control. This created a similar baseline situation before starting the bleaching, allowing a better comparison of the treatment effects among the groups.

Peroxide Penetration into the Pulp Chamber

Each specimen was placed inside a specially designed holder that kept the enamel/dentin disk over the artificial pulp chamber, as previously described (Figure 1D).[40,42,45] A perforated lid and a rubber O-Ring sealed the enamel surface, and protected the sides of the specimen from contact with the bleaching agents. Inside the chamber, 40 μL of 2M acetate buffer (pH 4.5) was placed to collect and stabilize the peroxide that permeated the tooth structure up to the moment of quantification. Using a positive displacement pipette (Microman E, model M100E, Gilson, Middleton, WI, USA), 20 μL of the bleaching agents (experimental and control) were applied over the enamel for 15 minutes inside a 100% relative humidity chamber at 37°C. After this time, the gel was removed with vacuum suction, and the application procedure was repeated twice for a total of 45-minutes of bleaching. The last gel was then removed with a small spatula and kept for further analysis, while the enamel surface was washed and dried.

The chambers were opened, and three samples of 5 μL of acetate buffer were collected and transferred into a 96-well microplate to analyze the peroxide concentration. The spectrophotometric analysis proposed by Bauminger[46] and modified by Hannig and others[47] based on the reaction of 4-aminoantipyrin and phenol with H_2O_2 catalyzed by peroxidase was used. A calibration curve was prepared in triplicate in the microplate using a standard 0.5 mM H_2O_2 solution. The absorbance was measured using a multimode microplate reader (Synergy HTX, Biotek) at a wavelength of 510 nm. The peroxide concentration was calculated in μg/μL, and the absolute means were used for the statistical analysis.

Post Bleaching Evaluations

The remaining bleaching gels, collected from the top of the specimens, were analyzed in relation to the peroxide concentration, pH, and ROS quantification. The enamel surface microhardness was immediately measured close to the area of the baseline readings. After that, the specimens were stored in 2 mL of artificial saliva inside the Eppendorf tubes for 14 days, with daily exchanges, using the formulation proposed by Klimek and others.[48] This storage period was necessary to complete rehydration of the specimens and release peroxide from the tooth structure, showing the actual bleaching effect obtained.

The color change between the baseline and posttreatment was calculated using the Δ*E*ab color difference equation.[49] Considering that for microhardness, peroxide concentration, and pH, the initial means were similar among the groups at the baseline (100%), the percentage of reductions was calculated by using the formula %Reduction = 100 – (Final Value × 100/Initial Value). For ROS, as the initial means were different among the groups, the absolute values were used for the analysis.

Statistical Analysis

The normality and homoscedasticity of the data were confirmed by the Shapiro–Wilk and Levene tests. The absolute baseline microhardness means, as well as the *L** and *b** coordinates were compared among the groups with one-way ANOVA. After the treatment, two-way ANOVA (viscosity × thickener) and the Tukey test were used for comparison among the groups for color change, peroxide penetration, percentage of microhardness reduction, peroxide, and pH reduction. For ROS, two-way repeated measures ANOVA (viscosity × thickener × time point) was performed. The Dunnett test was used for the comparison among the experimental groups with the negative control. For all analysis, the Statistica for Windows software program (StatSoft, Tulsa, OK, USA) was used, and a significance level of 5% was adopted.

RESULTS

One-way ANOVA showed no significant differences among the groups for the baseline values of microhardness (p=0.99), *L** (p=0.4487), and *b** (p=0.4327) color coordinates, showing that all groups had similar conditions before the treatment began.

Two-way ANOVA showed significant differences for viscosity and the kind of thickener for all analysis performed. The results of ANOVA and the Tukey tests for the factor "viscosity" are shown in Table 2. The high viscosity reduced the bleaching effect, peroxide penetration, softening of enamel, and ROS quantity in the gels in relation to the low viscosity. However, it increased the drop in pH and peroxide content in the bleaching gels.

The results of ANOVA and the Tukey tests for the factor "kind of thickener" are shown in Table 3. Gels produced with MSA showed higher color change in comparison with SSP and ASE, while the others showed no significant differences among them. The peroxide penetration was higher for PAC, and smaller for SSP and CAR. The microhardness reduction was higher for CAR and smaller for PAC. The higher pH reduction was seen for ASE and CAR, and the smaller for SSP. The higher peroxide concentration reduction was for SSP and the smaller was for CAR. The higher amount of ROS was for MSA and the smaller was for ASE.

In relation to the factor "time point" of measurement for ROS, a statistically significant reduction (p=0.0001) was observed from the beginning (17.08±8.02) to the end of the treatment (10.48±6.48).

The Dunnett test showed that the negative control group (water) had significantly smaller color change (ΔEab = 0.52±0.12) and percentage of microhardness reduction (%KHN reduction = −0.02%±0.006) in relation to the experimental groups. Also, there was a significant increase in pH (11.83%±2.39) in the negative control, while the experimental groups showed a reduction.

DISCUSSION

In this study, the viscosity of the bleaching gels and the kind of thickener significantly influenced all parameters analyzed, thus rejecting the null hypothesis. The higher the viscosity, the smaller the bleaching effect, the lower the peroxide penetration, the smaller reduction in microhardness and the number of free radicals in the gel. However, the pH and peroxide concentration reductions were higher for the more viscous gels (Table 2). A previous study reported the effect of the viscosity level (low, medium, and high) on the bleaching effect and pulpal penetration of a 10% hydrogen peroxide bleaching gel.[39] Although nonsignificant differences were observed among the groups in relation to bleaching, a higher penetration was observed for the low and medium viscosity (0.48 μg/mL and 0.44 μg/mL, respectively) in relation to the high viscosity (0.35 μg/mL),[39] consistent with the results of our study. Another study evaluated the effect of the viscosity of desensitizing gels on the pulpal penetration of potassium nitrate, also reporting a strong inverse correlation.[50] Of interest is why the increase in viscosity influenced the pulpal penetration of the active principles, either the peroxide[39] or the potassium nitrate[50] reported in those studies, or the hydrogen peroxide in the current study.

Before penetrating the tooth, the peroxide molecules in the gel layer applied on the enamel must leave the three-dimensional network formed by the thickener and permeate toward the gel–enamel interface. Those located at the boundary will leave the gel first, reducing the gradient of concentration in that area. The other peroxide molecules in the gel must diffuse from the more concentrated areas toward the less, as described in Fick's first law, passing through the maze formed by the thickener's chains until they reach the boundary, as they try to reestablish a concentration equilibrium. However, they are delivered rapidly, creating a flux of the active ingredients toward the tooth. As the peroxide concentration in the enamel is smaller than in the gel, the permeation is maintained during the whole treatment period. Therefore, the phenomena of internal diffusion of the drug, its release, and the

Table 2: *Means (SD) and Results of the Two-way ANOVA and Tukey Test for the Factor Viscosity*[a]

Viscosity	ΔEab	Penetration (μg/μL)	Knoop Hardness Number Reduction (%)	pH reduction (%)	% [HP] Reduction (%)	Reactive oxygen species
Low	3.27 (1.00) a	0.1451 (0.0126) a	−22.22 (4.40) a	−6.99 (2.34) a	−3.23 (0.92) a	16.24 (4.78) a
Medium	3.02 (0.84) b	0.1198 (0.0098) ab	−18.56 (5.42) b	−8.80 (4.88) b	−3.26 (0.91) b	13.82 (3.80) b
High	2.90 (0.78) c	0.1033 (0.0091) b	−15.39 (8.52) c	−9.22 (5.82) c	−4.41 (2.16) b	11.27 (2.18) c
ANOVA	0.0243*	0.0249*	0.0001*	0.0001*	0.0001*	0.0001*

Abbreviations: HP, hydrogen peroxide concentration.

[a]*Groups followed by different letters in each column present significant differences.*

**Significant differences according to ANOVA.*

penetration into the target tissue are all essential to the effect of the treatment.[11,16,51,52]

Hydrogen peroxide release by a bleaching gel has been reported to be higher for those with lower viscosity, although those with higher viscosity sustained release over longer periods.[20] Another study reported that drug release was inversely proportional to the concentration of the active ingredient and gel viscosity.[14] According to the authors, the differences may be related to the three-dimensional structure of the thickener network, where the liquid phase is maintained by capillary, adsorption, and molecular interaction.[14] Gels with thicker and more compact structure have a smaller network mesh that hinders free diffusion of drug molecules and consequently the release rate.[14] Other studies agree that the drug release of a gel is controlled by the complexity of the network formed by the thickener, which creates a long diffusion path for the active ingredient.[51,52] The solvent may be trapped in smaller thickener cells, acting as a diffusion barrier.[10]

The aim during the development of any formulation for topical application is to obtain the maximum flux of the active ingredient to the tissue to be treated instead of retaining the drug in the vehicle.[16] Among the critical factors affecting the flux is the concentration of the drug in the vehicle.[16] A higher peroxide concentration in the bleaching gel has been associated with higher pulpal penetration.[53,54] However, in the present study, although the peroxide concentration in the experimental gels was the same, permeation was reduced at higher viscosities, showing the importance of the vehicle in the flux and delivery of the active ingredient. It is important that the excipients (nontherapeutic ingredients) help obtain the target flux[16] instead of being an obstacle.

Although permeation of peroxide into the tooth structure is necessary for the bleaching effect, whether a higher penetration can increase the bleaching effect is still unclear.[4] Higher pulpal penetration has been reported to be unrelated to bleaching efficacy.[55] However, in our study, the less viscous gels showed higher bleaching efficacy and higher peroxide penetration. Additional studies on this topic should be performed to better understand this relationship. From a biological point of view, the increase in viscosity seems to be a good option to protect the pulp, while other methods such as chemical activation can improve the bleaching efficacy.[42,45,49,56] However, the present study did not simulate a positive outward pulpal pressure, which can strongly interfere with *in vivo* penetration.

Although studies evaluating low concentration peroxide gels for home bleaching concluded that the higher the viscosity, the slower the degradation of the peroxide remaining in the gel[57,58]; our study showed the opposite. The more viscous gels had increased reduction of peroxide concentration and of the ROS, which may be related to the higher amount of the base required to achieve the pH of the formulation. This may have destabilized the peroxide molecules, resulting in oxygen release instead of free radical formation,[59] which also may be associated with the higher pH drop in those groups (Table 2). The solvent evaporation rate and the consequent increase of the viscosity during the treatment can also interfere with the effect of the treatment[18,60,61] and may help explain the differences in peroxide concentration reduction among the groups. Additional studies are necessary to evaluate this effect during bleaching procedures, mainly for products where a single application is recommended by the manufacturer, and the gel is not reapplied.

In relation to the comparison among the thickeners, significant differences were observed for all the analyses. The synthetic polymeric thickeners carbomer (CAR) (Carbopol 980), alkali swellable emulsion (ASE) (Salcare SC 81), and modified sulfonic acid (MSA) polymer (Aristoflex AVC), as well the semisynthetic polymer (CMC) are polyelectrolytes, being similar to electrolytes (salts) and polymers (high-molecular-weight molecules), also known as polysalts.[9] They are available as particles formed by tightly coiled state molecules. For crosslinked thickeners, each particle can be considered as a network structure formed by many tightly coiled linear polymer chains interconnected by crosslinks, forming what can be considered a single, giant molecule. Without the crosslinks, the particles would be only a group of intertwined linear polymer chains but not chemically bonded.[62] After dissociation in water and neutralization, the acid groups become electrically charged. The repulsion among the similar charges modifies the three-dimensional configuration of the polymer chain to an uncoiled state, increasing the hydrodynamic volume. The entanglement and the weak bonding among some areas of the chains creates a network that immobilizes the solvent and increases the viscosity.

Aristoflex is an ammonium acryloyl dimethyl taurate/vp copolymer,[63] while Salcare and Carbopol are polyacrylates, which are copolymers containing a combination of acrylic acid, methacrylic acid, or its simple esters. CMC is produced from cellulose gum modified with carboxymethyl groups. They also have quite different molecular weight (Table 1). Therefore, the configuration of the network created is completely different, which may influence the internal diffusion of peroxide and its permeation into the tooth. Salcare and Carbopol have carboxyl groups (-COOH) that are protonated, creating an acidic medium after hydration,

but remaining in a coiled state.[63,64] To produce the ionization of the carboxyl groups and thickening effect, it is necessary to add base to increase the pH of the medium,[9] resulting in ionization and negative charges. As CMC and Aristoflex are salts, the acidic groups were previously neutralized by the manufacturer with sodium ($-CH_2CO_2Na$) and ammonia ($-CH_2SO_3NH4$), respectively. Undergoing ionization just after hydration, they release Na^+ or NH_4^+, respectively,[63,65,66] which can have some influence on the effect. Carbopol also showed the smaller reduction in peroxide concentration, which was consistent with previous studies.[57,58]

Although the thickening mechanism is similar, the Aristoflex AVC group showed increased bleaching compared to CMC or Salcare SC 81, which was also associated with more free radicals (Table 3). This can be related to the characteristics of the polymer network, since it has a higher molecular weight of around 10,000 g/mol, while the other two are smaller (262.19 and 314.37 g/mol) (Table 1). Another reason could be the release of $NHNH_4^+$ by the polymer, which may have interfered with the formation of free radicals. Additionally, Aristoflex required much less base (TEA) than all the other thickeners to reach the required pH, indicating the efficacy of the original neutralization. However, it produced one the highest microhardness reduction values, just below Carbopol, probably because of its high molecular weight and the great number of acidic groups in the molecule that have affinity with the calcium in the tooth structure.

The highest pH reductions during the period of treatment were observed for Salcare and Carbopol, which also required much more TEA than all the other thickeners because OH^- must be released from the base to induce ionization of the carboxyl groups. TEA ($C_6H_{15}NO_3$) is a tertiary amine and weak base of low toxicity that is frequently used to adjust the pH of gel formulations. After ionization, the anion OH^- will increase the pH, but the cation release will also be part of the gel and may interfere with the ionic interactions during the many reactions taking place. Both the cations and anions have been reported to be released by the bases used as pH conditioner in dental bleaching gels and interfere with the bleaching effect.[59] Another difference is that the Aristoflex and Carbopol are highly crosslinked and that, after hydration, they remain as a spherical particle of microgel, not as stretched molecules as for Salcare and CMC.[63,67,68] The expanded particles approximate each other, creating spherical globules and producing a hierarchical structure.[69] The higher the molecular weight and the crosslinking, the higher is the thickening effect.[63]

The Aerosil 200 thickener is basically composed of synthetic silicon dioxide particles of nanometric dimensions, also known as pyrogenic silica. It is insoluble in water or acid, and is not electrically charged.[9] When dispersed in a liquid, the particles form a network structure through hydrogen bonding of their surface silanol groups,[70] creating chain-like aggregates and producing a loose tridimensional network that immobilizes the solvent and increases viscosity.[9] Our results showed that the pulpal penetration was higher for Aerosil than for the other thickeners, probably because the peroxide released from this gel was also

Table 3: *Means (SD) and Results ANOVA and Tukey Test for the Factor "Kind of Thickener"*[a]

Thickener	Δ*E*ab	Penetration (μg/μL)	Knoop Hardness Number Reduction (%)	pH Reduction (%)	[HP] Reduction (%)	Reactive Oxygen Species
SSP (CMC)	2.80 (0.78) a	0.0613 (0.0089) a	–17.00 (4.01) c	–4.43 (1.68) a	–4.86 (1.85) c	9.76 (1.04) c
ASE (Salcare)	2.81 (0.80) a	0.1280 (0.0123) b	–14.71 (3.79) b	–13.87 (3.05) e	–3.53 (0.74) b	4.93 (0.82) a
PAC (Aerosil)	3.13 (0.80) ab	0.2069 (0.0090) c	–11.73 (3.98) a	–4.94 (1.42) b	–3.79 (1.36) b	10.88 (2.88) c
CAR (Carbopol)	3.24 (1.05) ab	0.0738 (0.0062) a	–28.06 (2.59) e	–12.62 (2.72) d	–2.50 (1.40) a	6.83 (1.55) b
MSA (Aristoflex)	3.32 (0.89) b	0.1435 (0.0096) b	-22.13 (4.43) d	-5.82 (2.41) c	-3.48 (1.21) b	16.48 (3.18) d
ANOVA	0.0071*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*

Abbreviations: HP, hydrogen peroxide concentration; SSP, semi-synthetic polysaccharide; ASE, alkali swellable emulsion; PAC, particulate colloids; CAR, carbomer; MSA, modified sulfonic acid polymer.

[a]*Groups followed by different letters in each column present significant differences.*

**Significant differences according to ANOVA.*

higher. An important problem with drug delivery is understanding the effect of the thickener matrix on the transport of low-molecular-weight active ingredients inside a gel. The transport conditions inside the network of uncharged thickener molecules, as in the case of silica particles, are expected to be similar to those inside the water.[12,71] However, drug and thickener chain interactions can occur if the gel is charged, giving rise to a non-Fickian transport.[12] In addition, the current study showed the smaller demineralizing effect for Aerosil, probably because of the absence of ionic interaction with the calcium in the tooth enamel, as seen for the other thickeners. The amount of TEA necessary to reach the required pH was smaller with higher gel viscosity, which was the opposite of the other thickeners. The pH reduction was smaller for this thickener than for Salcare and Carbopol, although the amount of ROS was higher.

In relation to the demineralization of enamel, the current study showed that the increase of the viscosity reduced its effects on the enamel, which may be related to the internal diffusion of the calcium captured by the thickener. In addition, all anionic thickeners tested showed a higher microhardness reduction than the nonionic one. The highest reduction was observed for Carbopol, followed by Aristoflex, which are both highly crosslinked and have higher molecular weights. Previous studies reported that Carbopol gels, even without peroxide, were able to reduce enamel microhardness[72–75] and increase surface roughness.[24] Calcium ions released by the enamel have been reported to permeate the unsaturated gels.[75,76] The demineralization of enamel and inhibition of hydroxyapatite crystal growth after exposure to Carbopol and CMC gels have been previously reported, even at neutral pH values, and were related to the negative charges of both the polyanions.[77] The polymers created complexes with the positively charged calcium ions and made the medium undersaturated.[77] The CMC also can strongly bond to multivalent ions such as Ca^{2+}.[67,78]

The negative control group showed significantly smaller values of color change and percentage of microhardness reduction in relation to the experimental groups, indicating that the method was sufficiently sensitive to detect even small color changes. The negative control also showed a significant increase in pH after application over the enamel (11.83±2.39%), while the experimental groups presented a reduction.

A bleaching effect from the thickener by itself was not expected, since its molecules cannot penetrate the tooth structure. Nevertheless, the absence of control groups evaluating the effect of peroxide free gels is a limitation of this study. Additional analysis is necessary to determine whether the best bleaching effect for some thickeners in the hydrogen peroxide gel formulation in this study may not be attributed to the thickener alone.

Based on our results, the effect of the viscosity and kind of thickener on the therapeutic and side effects of bleaching gels over the tooth structure was identified. The study of the vehicles of bleaching products is of high clinical significance, since most researchers are unaware that some aspects of the formulation may influence the results of the treatment more than the concentration of the active ingredient. Additional research is necessary to fully understand all aspects influencing the performance of the gels to create better and safer bleaching products.

CONCLUSIONS

The increase in bleaching gel viscosity reduced the color change, number of free radicals, peroxide permeation (PP), and demineralization of enamel, but increased peroxide decomposition and pH alteration during the treatment. The kind of thickener significantly interfered with the effect of the treatment on the tooth and chemical alterations in the gel during its application.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the animal subjects' oversight committee guidelines and policies of Institute of Science and Technology, Sao Paulo StateUniversity—UNESP. The approval code issued for this study is 05/11/2017.

Conflict of Interest

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

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Can Specular Gloss Measurements Predict the Effectiveness of Finishing/Polishing Protocols in Dental Polymers? A Systematic Review and Linear Mixed-effects Prediction Model

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Clinical Relevance

A clear and dependent relationship was found between specular gloss and roughness in resin composites. A reference value of >55 GU was found to be correlated with well-polished samples. This value can thus be used to objectively determine effectiveness of polishing and may serve as a starting point for future *in vivo* gloss measurements.

SUMMARY

Purpose: The current gold standard measure to assess polishing efficacy is surface roughness (SR) assessed in laboratory research. Specular gloss (SG) has been negatively correlated to SR, which raises the following question: Can SG be used to accurately

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determine the effectiveness of a finishing/polishing procedure in direct resin composites?

Methods: A systematic approach and search strategy, following Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines, was developed and conducted in five electronic databases: PubMed/Medline, Scopus, Web of Science, EMBASE (Ovid), and SciELO/ LILACS to identify laboratory studies that assessed SR and SG, simultaneously, of resin composites, without date or language restriction. Risk of bias assessment was carried out by two reviewers, independently. From the extracted quantitative data of SG/SR, regression analyses were performed, and a linear mixed-effects prediction model was derived using the nimble package in R (v4.0.3).

Results: A total of 928 potential studies were found, out of which, 13 were eligible after criterion screening. Experimental groups featured 31 resin composites of six different filler types, with the most common being microhybrids followed by nanohybrids. More than half of the studies initially reported a linear correlation between SR and SG, which ranged from r^2 = 0.34-0.96. Taking into account the regression analysis and prediction model posteriorly performed, the corresponding SG threshold for 0.2 μm is estimated to be >55 GU. Most of the evidence was classified as moderate or high risk of bias.

Conclusion: SG is universally correlated to SR in polymers, and a reference value of >55 GU is proposed, above which samples are considered well polished.

INTRODUCTION

Specular gloss (SG), like color, opalescence, translucency, and fluorescence, is a crucial parameter in dental aesthetics and is linked to the nature of the material, its surface properties, or external factors such as illumination and the observer itself.[1] Gloss is a parameter that comes from the geometrical distribution of light reflected on the surface of a material.[2] The distinction between a natural tooth and a restorative material can be found by measuring the difference in SG. This highlights the importance of gloss as a biomimetic parameter in restorative dentistry.[1,3] SG is capable of altering color perception.[4] Loss of gloss results in aesthetically unpleasant restorations, which makes them noticeable when adjacent to natural teeth.[5] Gloss variability also exists in restorative materials and especially in the natural tooth due to anisotropic texture in dental surfaces.[6]

Previous studies that determined SG in resin composites correlated it with surface properties such as SR.[4,7-10] This correlation is usually established in studies where different finishing/polishing systems are tested in order to assess the one which is the most efficient. A decrease in SR is linked with an increase in gloss in a correlation that some authors report as linear and inversely proportional.[9,11] SR of a tooth or restorative material has a direct impact on other important factors, such as dental plaque accumulation, by increasing the potential of microbial adhesion.[12] Thus, a smooth surface is crucial in maintaining periodontal health and avoiding events such as caries recurrence.[13,14]

A well performed finishing and polishing procedure is essential for the overall aesthetics of a direct restoration. It allows the manufacture of mirror and shadow areas, responsible for the way light is reflected by the enamel, playing a role in size and contour perception.[1,7] The existence of a reproducible, well-defined, simple, and predictable polishing sequence is therefore required to achieve acceptable smoothness and gloss, mimicking a natural tooth.[15] Great variability is present in polishing protocols, and evidence shows that systems that appear similar do not achieve comparable SR values.[8,16] Additional valid methods for polishing effectiveness are required.

Currently, the gold standard method to evaluate the effectiveness of a finishing/polishing protocol of a resin composite is the measurement of its SR. *In vivo* perception of roughness and visual gloss perception can be highly subjective and innacurate.[1,16] Both depend on the observational ability of the clinician and the patient's self-perception. The aim of this systematic review was to find whether gloss determination can be a valid alternative to evaluate the effectiveness of a polishing procedure of direct restorations and whether a minimum threshold gloss value can be determined to assess if samples are well polished. This review also seeks to examine the different types of SG measurements that have been conducted to assess finishing/polishing of resin composites, to investigate the variability in SG determination protocols, its correlation with SR, and what is still lacking in the evidence.

METHODS AND MATERIALS

Systematic Search

This systematic review followed Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines,[17] and the protocol, submitted to PROSPERO, was published as a preprint in

OSF open platform, following recommendations for protocols pertaining to *in vitro* studies that cannot be registered. The resulting protocol can be found at https://osf.io/4kvcb/ The systematic search was performed independently by two reviewers (TPM and AD). Search strategies were employed in the following five electronic databases: PubMed (MEDLINE), Scopus, EMBASE, ScieLO/LILACS, and Web of Science. The systematic search strategy included both Medical Subject Headings (MeSH) terms and free keywords. No language restrictions or publication date restrictions were applied in this review. The search included articles from inception until October 2020. The full electronic search strategy for PubMed/Medline was as follows: (((((((composite resins[MeSH Terms]) OR (resin composite)) OR (composite)) OR (restoration)) OR (dental resin[MeSH Terms])) AND ((((polish*) OR (finish*)) OR (dental polishing[MeSH Terms]) AND (((gloss*) OR (shin*)) OR (bright*)). The last search was conducted on November 5, 2020.

A hand search was also performed in the reference list of the papers that were eligible. Where full-text records could not be retrieved online, researchers were contacted via e-mail or digital platforms (researchgate.net).

Selection Criteria

Inclusion criteria—

- Laboratory, preclinical studies
- Studies with at least one experimental group that features direct resin composite as a restorative material
- Studies that have evaluated SG and SR simultaneously
- Studies that include finishing/polishing protocols that are clinically applicable and reproducible
- Studies that feature a control group (positive or negative) OR a baseline measurement before the polishing protocols
- Studies that evaluated SR using profilometer or AFM methods

Exclusion criteria—

- Studies that evaluated indirect materials such as ceramics, Computer-aided Design-Computer-aided Manufacturing (CAD-CAM) polymers, or indirect resin composite
- Studies that have evaluated other direct materials such as glass ionomer cements (GICs), resin-modified glass ionomer cements (RMGICs), or compomers
- Studies that have performed abrasion protocols (ie, with toothbrushes)
- Studies that used only standard laboratory polishing protocols (ie, SiC abrasion carbide paper) that are not clinically applicable
- Clinical studies

The defined intervention for this review were studies that evaluated finishing and polishing procedures with different systems in at least one experimental group with direct resin composite as a restorative material. The primary outcome evaluated was SG measurement [in gloss units (GU)], usually assessed by means of a glossmeter. The secondary outcome was SR determination—R_a (in μm or nm).

Screening of Primary Studies , Data Extraction, and Synthesis

Careful screening of the title/abstract from the references retrieved from the databases was carried out by two researchers (TPM and AD), independently, using Mendeley Desktop (v1.19.4). Papers that respected the inclusion criteria were considered eligible. After this stage, the full-text was acquired and evaluated. Disagreements were debated, and consensus was reached by seeking other review members (AMA and LL). At the full-text reading stage, reasons for exclusion were documented. As mentioned above, the primary studies also underwent a reference hand search.

A data extraction spreadsheet was developed and validated by five reviewers (TPM, AD, LL, SG, and PV). This form contained key information such as author/date, intervention type, experimental groups, sample size, material and classification, finishing/polishing system, how SR was measured (equipment/parameters), if a correlation of gloss with SR was found, and the study conclusions. Quantitative data such as SR and SG measurements were also extracted, in the form of means and standard deviations. Authors were contacted to provide access to datasheets when these were not available online. When this failed, bar charts were uploaded onto a digital platform for mean and standard deviation extraction (https://automeris.io/WebPlotDigitizer/). Data extraction was conducted independently by two reviewers (TPM and AD). When discrepancies existed, consensus was reached by consulting another review team member (LL, AMA and PV).

Quality Assessment—Risk of Bias

To assess the quality of the laboratory studies included, a risk of bias measurement was undertaken, following the method of prespecified risk of bias tools published in laboratory studies in dentistry.[18] The following criteria

were assessed: Randomization of samples, sample size calculation, presence of a control group, materials used according to manufacturer's instructions, appropriate outcome assessment, blinding of the outcome assessment, and correct reporting of outcomes. A YES/NO scale was used for classification. Classification was based on the number of "No" scores, with moderate risk being three parameters and high risk being more than three. The risk of bias plot was built using an online visualization tool - RoBvis 2.0 (https://mcguinlu.shinyapps.io/robvis/).

Statistical Analysis

Quantitative results from primary studies were pooled to originate datasets for SR and SG measurements of resin composites. These were used to model the relationship between both the variables. A sensitivity analysis excluded quantitative data from Lolita and others (2020)[19] due to significant outliers, Kamonkhatinkul and others (2014)[20], and Lopes and others (2018).[8] The estimates were derived using Markov chain Monte Carlo (MCMC) methods within the nimble package (v0.10.1) in R (4.0.3).

Bayesian Linear Mixed Effects Model

A normal hierarchical regression model, also called a linear mixed effects model, was used to describe the within-study heterogeneity of observations followed by the between-study heterogeneity, using a sampling model for the study-specific regression parameters.

The within-study model is:

$$y_{i,j} = \gamma_j^{\mathrm{T}} \boldsymbol{x}_{i,j} + \epsilon_{i,j}, \quad \epsilon_{i,j} \overset{iid}{\sim} N(0, \sigma_j^2) \quad (1)$$

where $x_{i,j} = (1, x_{i,j,1}, x_{i,j,2,\ldots})$ is a design vector representing the observed SR and other possible covariate values for the observation i in study j; where $j \in = \{1,2,\ldots, N=10\}$ and $i \in = \{1,2,\ldots, n_j\}$.

The heterogeneity among the regression coefficients, $y_1, \ldots, y_N$, will be described with a between-study model. Studies were modeled as exchangeable, assumed to be independent and identically distributed (iid) from some distribution representing the sampling variability across studies. Studies were modelled as exchangeable, ie considered as iid from some distribution representing the sampling variability across studies. The between-studies sampling model can be rewritten as:

$$\gamma_j = \boldsymbol{\beta} + \boldsymbol{b}_j \quad (2)$$

$$\boldsymbol{b}_j \sim N_2(\mathbf{0}, \Sigma) \quad (3)$$

which, transferred to the within-study regression model gives:

$$y_{i,j} = \beta_1 + \beta_2 x_{i,j,1} + b_{j,1} + b_{j,2} x_{i,j,1} + \epsilon_{i,j}. \quad (4)$$

In this parameterization, β is referred to as a vector fixed (population) effect as it is constant across studies, whereas b_j are called random effects, as they vary and are study-specific. "Mixed effects model" means the regression model that contains both fixed and random effects.

Given a prior distribution for β, σ^2, Σ, and having retrieved the SR and study, the observed data for the jth study are $D_j = \{(y_{i,j}, x_{i,j})\}_{i=1}^{n_j}$, the Bayesian analysis proceeds by computing the posterior distribution $p(\beta, b, \Sigma, \sigma^2|D)$, where D is the set of all data. This posterior distribution is approximated quite easily with Markov chain Monte Carlo (MCMC) methods within the nimble package in R. The prior distributions used are given in Table 1.

Bayesian Inverse Regression

Taking into account lower SR translates into polishing success, to correlate with SG, this problem, in its simplest form is one of inverse regression.[21] Rather than predicting SG values for a given SR, the aim was to invert this relationship to provide a prediction of SR to a specified SG value. The distributions required are for the entire population (10 studies).

Dropping the subscripts in the model, writing of the mean SG value is possible as $E[\Upsilon_0]$ at roughness x0 as $E[\Upsilon_0] = \alpha_0 + \alpha_1 x_0$. Inversion of this relationship produces the desired roughness for a specified $E[\Upsilon_0]$; that is $x_0 = (E[\Upsilon_0] \alpha_0)/\alpha_1$. Such distributions were summarized in terms of quantiles. Posterior features for the population parameters can be seen in Table 2.

Exploratory Analysis

To deal with the small values of the roughness and to be able to consider a normal distribution for the gloss values, a logarithmic transformation of both variables was undertaken.[22] In Figure 1, it is possible to discern

Table 1: *Prior Distributions Considered for Each Parameter in the Application*[a]

Parameter	Prior Distribution
β_1	$N(0, \sigma_{\beta 1})$
β_2	$N(0, \sigma_{\beta 2})$
$\sigma_{\beta 1}, \sigma_{\beta 2}$	$U(0.001, 100)$
$b_j \Sigma$	$N_p \geq 2(0, \Sigma)$
Σ^{-1}	*Wish*(diag(100), 2)
σ_j	$U(0.001, 100)$

[a] *The notation considered for each distribution is N (., .), N_p(., .), Wish(., .) and U (., .) standing respectively, for univariate normal, p-variate normal, Wishart, and uniform.*

Table 2: *Posterior Features for the Population Parameters (Means and 95% Credibility Intervals Shown)*

Parameter	Mean	95% Credibility Interval
β_1	2.326	(1.536, 2.986)
β_2	-0.690	(–0.953, –0.469)
$\sigma_{\beta 1}$	30	(1, 100)
$\sigma_{\beta 2}$	20	(10, 100)

that within each study, the higher the log(*SR*) the lower the log(*SG*). However, there is some common trend able to be retrieved by considering the Bayesian hierarchical model defined in (4). Note the high within- and between-study variability, and a clear indication of a decreasing trend. There are indications that the studies produce very different results whether looking at SR or SG.

RESULTS

Systematic Search and Data Retrieval

The systematic search retrieved 928 references in total from the five databases in which the search was conducted. After title and abstract screening, 460 references were excluded and 1 additional reference was found during manual searching. 32 final references were eligible for full-text access. Out of these, 19 studies were excluded: 12 did not have a control group or a baseline measurement of SR, and/or SG, 4 did not use finishing/polishing systems with clinical applicability, and 3 did not directly measure and determine gloss. A summary of the PRISMA flowchart can be seen in Figure 2.

The remaining 13 studies were eligible for synthesis in this systematic review, and extracted data are shown in Table 3. All the studies included measured SG and roughness in direct resin composites, with different intervention aims, using a laboratory study design. Only the experimental groups containing resin composites were featured for data synthesis and are shown in the table.

Experimental Groups: Resin Composite

A total of 31 resin composites was evaluated in the 13 studies included in this review. Only 2 studies evaluated individual materials, by varying only the finishing/polishing protocol,[10,19] while the remaining 11 studies compared different resin composites with distinct filler classifications.[8,20,23-31] Regarding filler classification, the preferred filler type studied was microhybrids (15/31 groups): ceramX, Clearfil AP-X, Clearfil Posterior, Enamel Plus HFO, Esthet X, Grandio, Filtek Silorane, Filtek Z250, FZ-Dentin, FZ-Enamel, Premise, Prisma APH, Tetric EvoCeram, Tetric Ceram, Venus; followed by nanohybrids (9/31): Brilliant Everflow, Clearfil Posterior, Estelite Asteria, IPS Empress Direct, Kalore, Sonic Fill 2, Tetric EvoFlow, Venus Diamond, Venus Pearl; nanofilled (3/31): Filtek BulkFill, Filtek Supreme XTE and Filtek Z350; and finally microfilled (3/31):

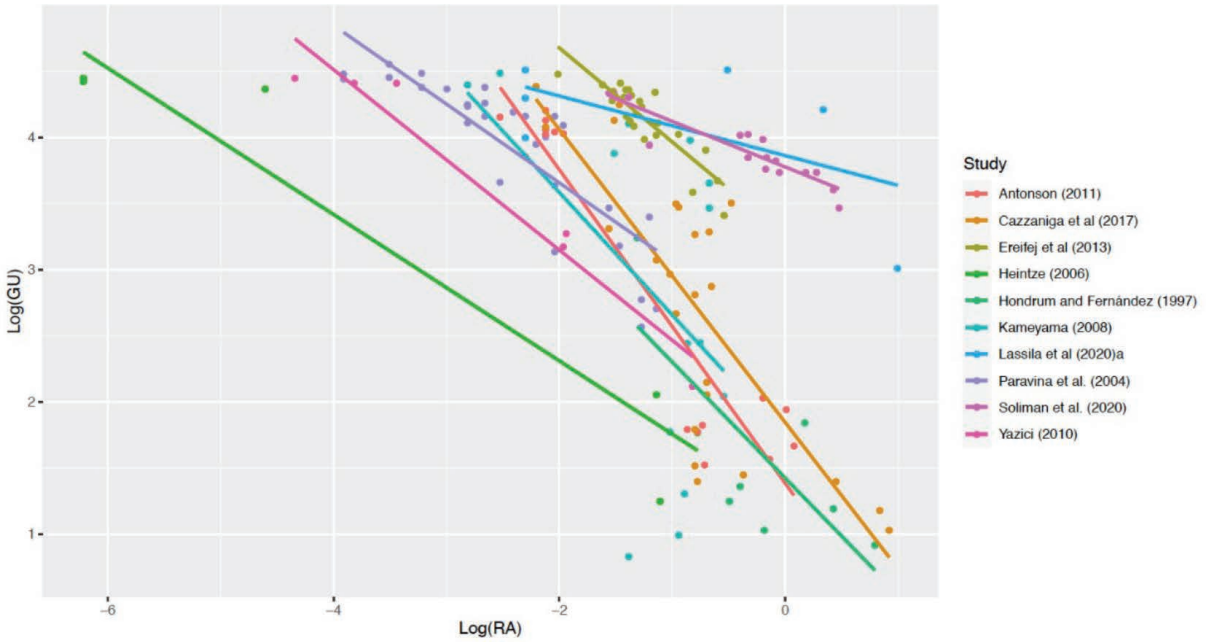

Figure 1. *A linear model fitted to each individual study.*

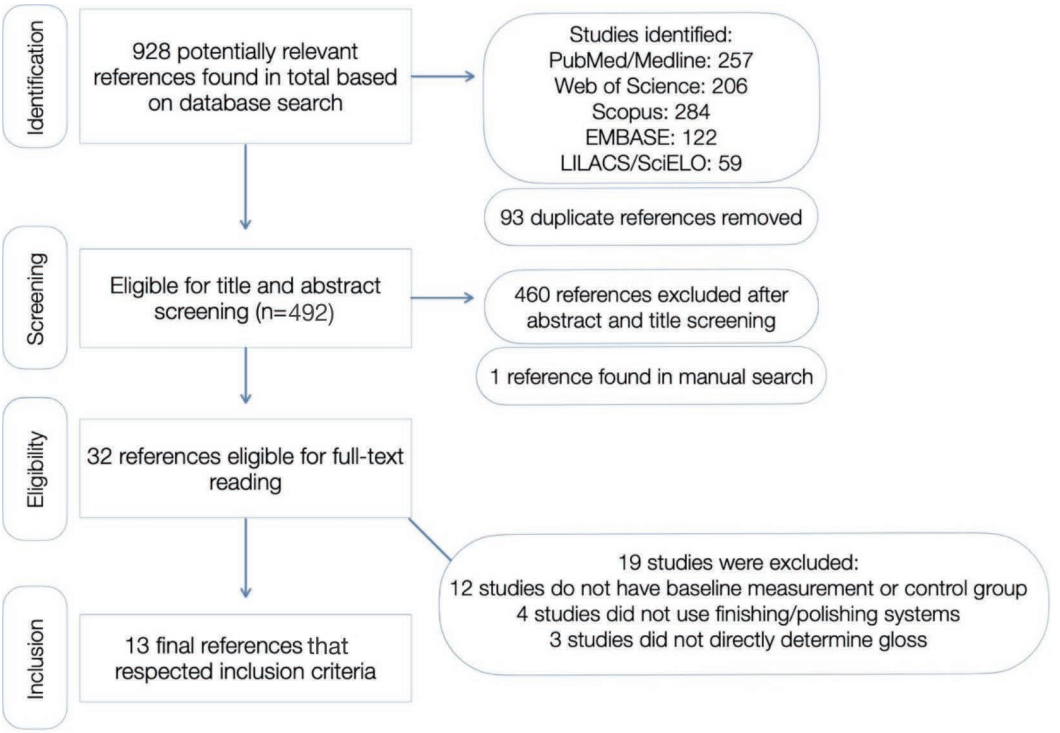

Figure 2. Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) flowchart followed in this systematic review.

Durafill, Heliomolar RO and Renamel Microfill. One submicron resin composite (Estelite Σ) was also studied. Paravina and others (2004) studied two experimental microhybrid composites, while the rest of all studied commercial products.[31]

Experimental Groups: Finishing and Polishing Systems

Most studies compared more than two finishing and polishing systems (11/13), and only two studies compared an individual polishing system.[28,30] A Mylar strip was used in seven studies as a positive control for low SR and high SG.[10,19,23,25,28,31] Sof-Lex discs were the most common polishing system evaluated (8/13), followed by finishing burs (4/13), and the Enhance PoGo system (4/13). Silicon carbide paper was also used to provide varying degrees of roughness, or as a control, in three different studies.[10,20,26] Transversal to all studies, the smoothest and the glossiest surfaces were produced with a Mylar strip when it was used as a control. All the studies support SG and SR as being material dependent and polishing-protocol dependent.

Outcomes

Surface roughness—Surface roughness (SR) was assessed using traditional surface profilometers[19,20,23-25,27,29,31] and 3D noncontact profilometers.[26,28,30] The measurements varied from one to five line tracings/scans per sample. Soliman and others (2020) used an environmental SEM and software analysis, while Lopes and others (2018) used an AFM.[8,24]

Surface gloss—To assess SG in the studies, six different glossmeters were used. The preferred glossmeter was a small area device supplied by NovoCurve (6/13 studies), while the remaining all used different devices, with varying measurement areas. All SG measurements were done at a 60° angle, and three to five measurement repetitions were performed for each sample.

Correlation Between Outcomes

Generally, when SR decreased, an increase in SG was noted, which justifies a negative linear relationship. A correlation between SR and SG was determined in 8 of the 13 studies included (64%). The strength of the correlation varied from $r^2 = 0.34$ to $r^2 = 0.96$ and was found to be material dependent, with results varying between experimental groups when subanalyses were carried out, as reported in the study of Cazzaniga and others (2017).[25] Additionally, two studies determined a correlation but failed to report it.[19,24]

Model Results

The assessment of convergence and mixing of chains within the MCMC approach has been carried out

by looking at plots of autocorrelation and trace of the chains. Several chains were generated starting from different initial values, and all provided a rough indication of convergence after a small period. A numeric diagnostic Gelman-Rubin (GR) has also been considered, revealing no concerns.

Table 4 gives posterior means and interval estimates in the form of 95% equal-tail credible sets for the common effect, β_1, as well as for the roughness effect, β_2. Study-level parameter estimates are summarized in Table 4. The column corresponding to σ_j shows a relative low variability; on the log scales, the within-study variabilities are reasonably homogeneous across studies, yet not equal. The set of all study-level slope effects, $b_{j,2}$, suggests a large between-study variability. A negative correlation between the individual intercepts and slopes was found, as explained by $\Sigma_{1,2} = \Sigma_{2,1} = -0.2$.

Concerning the inverse prediction, to answer the main question, simulated scenarios can be seen in Table 5. Simulations were carried out using SG values spanning from 0.5 to 4.5 on the log scale, since values in the dataset have a minimum of 0.8 and a maximum of 4.5 on the log scale.

A roughness threshold of 0.2 = μm was considered for analysis or on the log scale log(2) = −1.609. Thus, log(SG) values indicating a roughness lower than −1.609 were sought. From Table 4 and Figure 3, it is possible to infer that at a log(GU) < 3.5 there is about 60% probability of having a SR lower than the threshold. With a log(*Gloss*) 4, all probability intervals have their boundaries below 1.6, indicating a SR below threshold (p~1).

Quality Assessment—Risk of Bias

The risk of bias assessment and judgment of each parameter is summarized in Table 6. The majority of studies (46%) were classified as having high risk of bias, followed by 38% with moderate risk of bias. Two studies (15%) were low risk. None of the studies included performed *a priori* sample size calculation, and all presented a control group or baseline measurement. Weighted plot summary is shown in Figure 4.

DISCUSSION

The purpose of this study was to determine whether SG measurement is a valid method of evaluating the effectiveness of a polishing procedure of direct resin composites, and, also, whether a minimum threshold gloss value can be proposed to inform clinicians and researchers that the samples/restorations are properly polished. To this day, no systematic review or subsequent analysis was found that determined the correlation between both these variables, taking into account the pooled results, by studying their codependence. The model presented in this study answered the question posed by confirming that SG can correlate with SR values, and thus effectively evaluate a polishing procedure.

This review included only direct resin composites. A direct restorative procedure is more subject to variability and difficulty in finishing and polishing procedures, making it clinically relevant.[16,32] Laboratory manufactured resin and ceramic restorations are produced in a controlled environment, less susceptible to protocol variations, and may be subject to additional gloss-producing measures, such as glazing.[33] Filler type is believed to be of paramount importance for final smoothness and gloss of composites. The majority of resin composites studied were microhybrids, which is not surprising, as universal restorative composites are of microhybrid filler type.[34] In what concerns aesthetic restorations, there is a common misconception that a resin composite with filler particles in a smaller range should be used, as there would be less changes in surface characteristics from wear-induced loss of filler particles. This influenced the industry into producing nanohybrid and nanofilled resin composites.[34,35] Kaizer and others (2014), however, in a systematic review, found that there is no significant influence of filler type on SG and SR.[35]

Only one study included in this review evaluated SG and SR of bulk-fill resin composites. These materials represent a significant innovation.[10] Since they allow us to reduce chair time, they are becoming increasingly popular among professionals and widely used, especially in posterior restorations. Thus, surface quality of these composites should be further evaluated in order to validate them in these parameters for generalized clinical use, here having the main purpose of minimizing biofilm accumulation and the recurrence of secondary caries, as they are generally used in posterior, nonaesthetic areas.[36,37]

This systematic review included only studies with a control group or a baseline measurement of SR and SG. As a positive control, most of the studies used a Mylar strip, as it is widely accepted, this method produces the lowest SR and the highest SG, comparatively. Conversely, as a negative control, silicon carbide papers of smaller grit size or abrasive burs were used. A negative control will illustrate the effect of only performing finishing procedures on restorations without polishing of any sort, which was proven insufficient for attaining acceptable SG and SR on all the studies that used this type of control. Furthermore, finishing procedures should always be followed by final polishing.[12]

Table 3: *Summary of Studies Included in the Systematic Review*

Author	Intervention	Sample Size	Resin Composite	Finishing/Polishing System
Lassila and others (2020)[10]	Effect of different finishing/polishing systems and curing modes on SR and SG of one resin composite	n=3	Filtek BulkFill (3M, St Paul, USA) - nanofilled	Mylar strip 1200grit SiC 2400 grit SiC 4000 grit SiC Sof-Lex spirals (3M, St Paul, USA) 2 step Jiffy Polishing points (Ultradent, St. Louis, USA) 1 step
Soliman and others (2020)[24]	Effect of different finishing/polishing systems on SR and SG of different resin composites	n=7	IPS Empress Direct (Ivoclar Vivadent, Schaan, Liechtenstein) nanohybrid Grandio (Voco) nanohybrid Filtek Z550 (3M, St Paul, USA) nanohybrid Filtek Z250 (3M, St Paul, USA) microhybrid	Optrapol (Ivoclar Vivadent, Schaan, Liechtenstein) 1 step Politip (Ivoclar Vivadent, Schaan, Liechtenstein) 2 step Sof-Lex discs (3M, St Paul, USA) 3 step
Lolita and others (2020)[19]	Effect of different finishing/polishing systems on the SR and SG of a nanoceramic resin composite	n=5	ceramX. SphereTec Universal (Dentsply Sirona, Charlotte, USA) nanohybrid	Mylar strip Enhance (Dentsply Sirona, Charlotte, USA) 1 step Diacomp Twist spirals (EVE, Keltern, Germany) 2 step Sof-Lex discs (3M, St Paul, USA) 4 step
Lopes and others (2018)[8]	Effect of different finishing/polishing systems on the SR and SG of two different resin composites	n=5	Brilliant Everglow (Coltene/ Whaledent, Altstätten, Switzerland) nanohybrid Filtek Supreme XT (3M, St Paul, USA) nanofilled	Sof-Lex discs (3M, St Paul, USA) 2 step Sof-Lex spirals (3M, St Paul, USA) 2 step SwissFlex Disc 2 step DiaTECH burs (EVE, Keltern, Germany) 2 step Enhance cups 2 step Diashine Polishing compound and suede disc (EVE, Keltern, Germany) 2 step Finishing abrasive bur

Table 3: *Summary of Studies Included in the Systematic Review*

SG Measurement	SR Measurement	Correlation Between Variables	Conclusion
Calibrated infra-red Zehntner-Glossmeter at 60° - 6x40 mm area	Surface profilometer using five profilometer tracings	Linear correlation determined (r^2=0.938)	The smoothest surfaces were obtained with laboratory polishing (4000 grit). Polishing protocols had an effect on the SG and SR
Glossometer (PICOGLOSS 560MC) at a 60° angle	Image analysis software together with environmental scanning electron microscope	Correlation analysis determined but not reported.	The multi-step system seems to be more effective on SR and SG results. Both variables were significantly influenced by filler type and finishing/polishing system
Small area gloss meter (NovoCurve) at 60° - 2x2 mm area	Surface profilometer using five profilometer tracings	Correlation analysis determined but not reported.	The four-step polishing system resulted in the highest SG and lowest SR
A glossmeter (Micro-Tri-Gloss 4520) was used at 60° over a 2x4 mm area	AFM was used to analyse the central region. 80 sections of 10x10 um were analysed per polishing protocol	Linear correlation determined (r^2=0.419)	Type of polishing system and resin composite influences the results. SG results in composites are related to surface roughness anisotropy and its conjoined effects.

Table 3: *Summary of Studies Included in the Systematic Review (cont.)*

Author	Intervention	Sample Size	Resin Composite	Finishing/Polishing System
Cazzaniga and others (2017)[25]	Effect of surface treatments on the microbial adhesion of different resin composites	n=5	Enamel Plus HFO (Micerium, Avegno, Italy) microhybrid Estelite Asteria (Tokuyama, Yamaguchi, Japan) nanohybrid Filtek Supreme XTE (3M, St Paul, USA) nanofilled Sonicfill 2 (KaVo/Kerr, Brea, USA) nanohybrid	Mylar strip Sof-Lex discs (3M, St Paul, USA) 3 step Opti1Step (KaVo/Kerr, Brea, USA) 1 step Diamond bur Multi-blade carbide bur
Kamonkhatinkul and others (2014)[20]	Effect of finishing/polishing and toothbrushing cycles on the SR and SG of different resin composites	n=6	DuraFill (Heareus Kulzer, Hanau, Germany) microfilled Filtek Z250 (3M, St Paul, USA) microhybrid Filtek Z350 XT (3M, St Paul, USA) nanofilled Kalore (GC Corporation, Tokyo, Japan) nanohybrid Venus Diamond (Heraeus Kulzer, Hanau, Germany) nanohybrid Venus Pearl (Heraeus Kulzer, Hanau, Germany) nanohybrid	2400 and 4000 grit SiC Sof-Lex discs (3M, St Paul, USA) 1 step Venus Supra discs (Heraeus Kulzer) 1 step
Ereifej, Oweis, and Eliades (2013)[26]	Effect of different finishing/polishing systems on SR and SG of different resin composites	n=5	Filtek Silorane (3M, St Paul, USA) microhybrid IPS Empress Direct (Ivoclar Vivadent, Schaan, Liechtenstein) nanohybrid Clerfil Majesty Posterior (Kuraray, Tokyo, Japan) nanohybrid Premise (KaVo/Kerr, Brea, USA) microhybrid Estelite Sigma (Tokuyama, Yamaguchi, Japan) submicron	Control (320 grit to 4000 grit SiC) Opti1Step (KaVo/Kerr, Brea, USA) 1 step OptiDisc discs (KaVo/Kerr, Brea, USA) 3 step Kenda discs 3 step PoGo micropolisher disc (Dentsply Sirona, Charlotte, USA) 1 step

Table 3: *Summary of Studies Included in the Systematic Review*

SG Measurement	SR Measurement	Correlation Between Variables	Conclusion
Small area gloss meter (MG6-SA; KSJ) at 60° - 2x2 mm area	Surface profilometer using three line tracings	Linear correlation determined r^2 [0.336-0.542]	The one-step system showed the highest SG values. The correlation between SR and SG was material dependent and highest for the microhybrid composite.
Calibrated glossmeter (IG-331, Horiba) at a 60° - 3x6 mm^2 oval shaped area	Surface profilometer using five parallel tracings in two perpendicular directions	Linear correlation determined r^2=0.63	Both systems produced a high SG on the resin composites tested. Toothbrushing up to 40k cycles caused a significant decrease in SG and increase in RA, except for VEP and Z350 in Ra.
Measured with a glossmeter (NovoCurve) at 60° - 2x2 mm area	Noncontact 3D optical interferometric profilometer one scan per sample surface	Linear correlation determined r^2=0.871	One-step polishing systems resulted in better surface finish for the resin composites tested. The best polishing system is material dependent. The two variables had an impact on the SR and SG

Table 3: *Summary of Studies Included in the Systematic Review (cont.)*

Author	Intervention	Sample Size	Resin Composite	Finishing/Polishing System
Antonson and others (2011)[27]	Effect of different finishing/polishing systems on SR and SG	n=5	Esthet X (Dentsply Sirona) microhybrid Filtek Supreme XTE (3M, St Paul, USA) nanofilled	Astropol (Ivoclar Vivadent, Schaan, Liechtenstein) 3 step Enhance PoGo (Dentsply Sirona, Charlotte, USA) 1 step Sof-Lex discs (3M, St Paul, USA) 3 step EXL 695 discs (3M) 2 step
Yazici and others (2010)[28]	Effect of immediate and delayed polishing on SR and SG of different resin composites	n=10	Tetric Flow (Ivoclar Vivadent, Schaan, Liechtenstein) nanohybrid Venus (Heraeus Kulzer, Hanau, Germany) microhybrid Grandio (VOCO, Cuxhaven, Germany) microhybrid	Mylar strip Finishing was performed with 30 µm diamond finishing burs followed by Sof-Lex discs 3 step
Kameyama and others (2008)[29]	Effect of different polishing systems on SR and SG of different resin composites	n=5	Clearfil AP-X (Kuraray, Tokyo, Japan) microhybrid Estelite Sigma (Tokuyama, Yamaguchi, Japan) submicron	Diamond Point FG (Shofu, Kyoto, Japan) White Point CA Shofu, Kyoto, Japan Stainbuster (Danville Materials) Compomaster CA Shofu, Kyoto, Japan 1 step
Heintze, Forjanic, and Rousson (2006)[30]	The influence of the press-on and polishing time on the SR and SG of differentdental materials and the relationship between them	n=8	Heliomolar RO (Ivoclar Vivadent, Schaan, Liechtenstein) microfilled Tetric Ceram (Ivoclar Vivadent, Schaan, Liechtenstein) microhybrid Tetric Evoceram (Ivoclar Vivadent, Schaan, Liechtenstein) microhybrid	Astropol (Ivoclar Vivadent, Schaan, Liechtenstein) 3 step

Table 3: *Summary of Studies Included in the Systematic Review*

SG Measurement	SR Measurement	Correlation Between Variables	Conclusion
Small area glossmeter (NovoCurve) at 60° - 2x2 mm area	Surface profilometer using three line tracings at different locations	Not determined	Sof-Lex discs produced the lowest SR, while EXL-695 produced the highest gloss.
Small area glossmeter (NovoCurve) at 60° - 2x2 mm area	3D non-contact interferometric profilometer using three line tracings in different locations.	Not determined	The smoothest surfaces were produced with a Mylar strip. The effect of delayed finishing/ polishing on the SG and SR is material dependent
Precision glossmeter GM 260 (Murakami color research Laboratory)	Surface profilometer using five profilometer tracings	Linear correlation determined r^2 [0.80-0.88]	The polishing procedures produced an effect on SG and SR. There is no significant difference between SR of both materials, even though ES had higher gloss. There is a clear relationship between average SR and SG
Small area glossmeter (NovoCurve) at 60° - 2x2 mm area	3D non-contact optical profilometer using a 1x1mm^2 area	Linear correlation determined r^2 [0.91-0.96]	The higher press on force increased SR in resin composites. SG and SR were time-dependant and negatively correlated. SG assessment may be a sufficiently accurate method to determine the polishability of materials.

Table 3: *Summary of Studies Included in the Systematic Review (cont.)*

Author	Intervention	Sample Size	Resin Composite	Finishing/Polishing System
Paravina and others (2004)[31]	Effect of different finishing/polishing systems on SR and SG of different resin composites	n=4	FZ-Dentin experimental microhybrid FZ-Enamel experimental microhybrid Heliomolar RO (Ivoclar Vivadent, Schaan, Liechtenstein) microhybrid Esthet-X (Dentsply Sirona, Charlotte, USA) microfilled Renamel Microfill microfilled	Mylar strip Carbide finishing bur Astropol (Ivoclar Vivadent, Schaan, Liechtenstein) 3 step Sof-Lex discs (3M, St Paul, USA) 3 step PoGo micropolisher disc (Dentsply Sirona, Charlotte, USA) 1 step Enhance and PRISMA Gloss (Dentsply Sirona, Charlotte, USA) 2 step
Hondrum and Fernandez (1997)[23]	Effect of different finishing/polishing systems on SR/SG a resin composite, glass ionomer and resin-modified glass ionomer	n=7	PRISMA APH (Dentsply Sirona, Charlotte, USA) microhybrid	Mylar strip Sof-Lex Discs (3M, St Paul, USA) 3 step Two striper MFS/MPS system 2 step Composite Finishing System Enhance Finishing/Polishing System (Dentsply Sirona, Charlotte, USA) 2 step Two striper MPS Contouring Burs (7901 and 9714)

Abbreviations: 3D: three-dimensional; ES: Estelite Sigma SiC: Silicon carbide; SG: specular gloss; SR: surface roughness

Considering the finishing/polishing systems evaluated, Sof-Lex Discs (3M, St Paul, MN, USA) are a multistep system commonly used clinically and were one of the most used polishing systems in the studies included. These were able to achieve the high SG values and low SR, reaching the roughness threshold of 0.2 μm defined by Bollen, Lambrechts, and Quirynen in 1997[38] in some of the studies included in this review,[20,25,27,28,31] which was also supported by other researchers.[3] As previously found, SR is highly material dependent. In four of the five aforementioned studies, only the microhybrid resin composites attained values under the threshold for SR. The study by Khamonkhantikul and others, on the contrary, found SR values well under 0.2 μm in all the resin composites studied, including nanofilled and nanohybrid ones.[20] In line with previous findings, Lolita and others in 2020 studied a single nanoceramic resin composite, and polishing with Sof-Lex discs was not enough to obtain satisfactory SR values.[19] These results are contested by Kaizer and others in a systematic review that found that no evidence to support that filler type has an influence on such surface properties, by testing nanofill, submicron, and traditional microhybrid resin composites.[35] Over time, abrasion of the surface organic matrix with loss of filler leads to an increase in SR. Finishing/polishing such surfaces is able to return lower SR values, which also increases SG.[39] Achieving optimal SR of dental restorations is also important in mimicking natural enamel characteristics. While unpolished enamel may

Table 3: *Summary of Studies Included in the Systematic Review*

SG Measurement	SR Measurement	Correlation Between Variables	Conclusion
Small area glossmeter (NovoCurve) at 60º - 2x2 mm area	Surface profilometer which was used to measure four tracings at different locations	Linear correlation determined r^2= 0.77	The smoothest and shiniest surfaces were produced with the mylar strip, followed by the PoGo system. SG showed differences between the resin composites and SR was also material dependent.
Glossmeter (Model GM-060, Minolta Corp, Ramsey)	Surface profilometer which was used to measure lines perpendicular to the striations.	Correlation analysis determined but not reported.	The smoothest and shiniest surfaces were produced with the mylar strip. SG decreased while using rotary instruments.

range from 3 to 1 μm, polished enamel can reach values less than 0.5 μm, even 0.15 μm.[40,41] A mismatch of SR values between enamel and restorative materials will cause disparities in light reflectivity.[42]

Since there is a permanent search for simplified protocols in dentistry, single-step finishing/polishing systems such as Enhance PoGo (Dentsply Sirona, Charlotte, USA) were also evaluated but showed an unsatisfactory performance regarding the SR threshold range in all studies, except in those of Antonson and others, and Paravina and others.[27,31] These results lead us to believe that applying more complex multistep systems produces better results, as corroborated by the previous authors.[10,19,43] Contradicting these results, however, Paravina and others, Ereijef and others, and Cazzaniga and others, showed that the one-step polishing systems they tested performed better than the multistep.[25,26,31] This disparity of findings may be related to different protocols being followed in these investigations, even those using the same polishing systems. Some of the authors opted for skipping steps in complex systems. There was a lack of a systematized polishing time and pressure, RPMs at which the several steps were performed and overall use of water cooling, which may impact the results. Only one of the studies evaluated included polishing time as a variable.[30] It is important to lay out that this is a factor that will impact resulting SR and SG. The need for standardized protocols is, therefore, of the utmost relevance for any further studies and future clinical trials.

Table 4: *Posterior Features for the Study-specific Model Parameters*

Parameters	Mean	Confidence Interval	Parameters	Mean	Confidence Interval	Parameters	Mean	Confidence Interval
b1,1	-0.84	(-159, -0.02)	b1,2	-0.42	(-0.73, -0.10)	σ1	0.17	(0.09, 0.39)
b2,1	-0.38	(-1.12, 0.43)	b2,2	-0.28	(-0.65, 0.07)	σ2	0.4	(0.24, 0.75)
b3,1	0.95	(0.25, 1.75)	b3,2	-0.003	(-0.28, 0.30)	σ3	0.02	(0.01, 0.04)
b4,1	-0.96	(-1.83, 0.05)	b4,2	0.15	(-0.12, 0.46)	σ4	0.18	(0.07, 0.92)
b5,1	-0.85	(-1.60, -0.03)	b5,2	-0.17	(-0.59, 0.24)	σ5	0.24	(0.11, 0.86)
b6,1	-0.38	(-1.41, 0.68)	b6,2	-0.10	(-0.61, 0.38)	σ6	1.14	(0.61, 2.57)
b7,1	1.39	(0.52, 2.29)	b7,2	0.38	(0.03, 0.77)	σ7	0.2	(0.08, 0.93)
b8,1	0.13	(-0.61, 0.96)	b8,2	0.09	(-0.16, 0.37)	σ8	0.1	(0.06, 0.17)
b9,1	1.45	(0.78, 2,23)	b9,2	0.34	(0.10, 0.62)	σ9	0.02	(0.01, 0.05)
b10,1	-0.51	(-1.32, 0.37)	b10,2	0.01	(-0.26, 0.32)	σ10	0.07	(0.03, 0.3)
						Σ1,1	0.43	(0.20, 1.34)
						Σ1,2 = Σ2,1	-0.20	(-3.70, -0.08)
						Σ2,2	0.04	(0.02, 0.14)

Gloss consists a variety of surface phenomena that represent the ability of light reflectance of a surface, rather than one single parameter.[44] SG can be measured at standardized angles of 20°, 60°, and 85°.[1] Past literature and ISO 2813, ASTHD 523 and 2457, and DIN 67530, describe the 60° angle geometry as a general standard for moderate gloss values. At the extremes (<10 and >70 GU), it behaves nonlinearly, which stresses the need to use other geometries. The perception of gloss variations by different observers is also impaired in these extremes.[1] This explains why some values are indistinguishable at higher and lower SR values, following an exponential decay.[1,45]

In contrast to 20° and 85° angles, 60° angle measurement is the one that falls closest to the angle from which the average individual will observe the surface.[3]

Previous studies that evaluated different materials, such as photographic paper, chocolate, egg-shells, and metal finish, found strong correlations between surface texture and gloss reflectance.[46-49] Heintze and Zimmerli reported an exponential function to explain the correlation between SR and SG in dental resin composites.[50] Furthermore, these studies classified the relationship as an exponential increase in SG for a decrease in SR, especially concerning higher SG

Table 5: *Inverse Prediction—Posterior Quantiles for the Distribution of the Mean SR Value Considering SG in the First Column*

		log(RA): Quantiles					
Gloss	Log(SG)	0.025	0.25	0.5	0.75	0.975	P[log(RA)≤1.609]
1.65	0.5	1.17	2.17	2.71	3.28	4.91	0
2.72	1	0.61	1.50	1.97	2.47	3.93	0
4.48	1.5	0.04	0.84	1.23	1.66	2.90	0
7.39	2	–0.54	0.16	0.50	0.86	1.90	0
12.18	2.5	–1.12	–0.52	–0.24	0.08	0.92	0
20.09	3	–1.76	–1.22	–0.97	–0.70	–0.03	0.0466
33.12	3.5	–2.51	–1.95	–1.70	–1.45	–0.91	0.5946
54.60	4	–3.32	–2.69	–2.43	–2.18	–1.70	0.977
90.02	4.5	–4.21	–3.46	–3.16	–2.89	–2.41	0.977

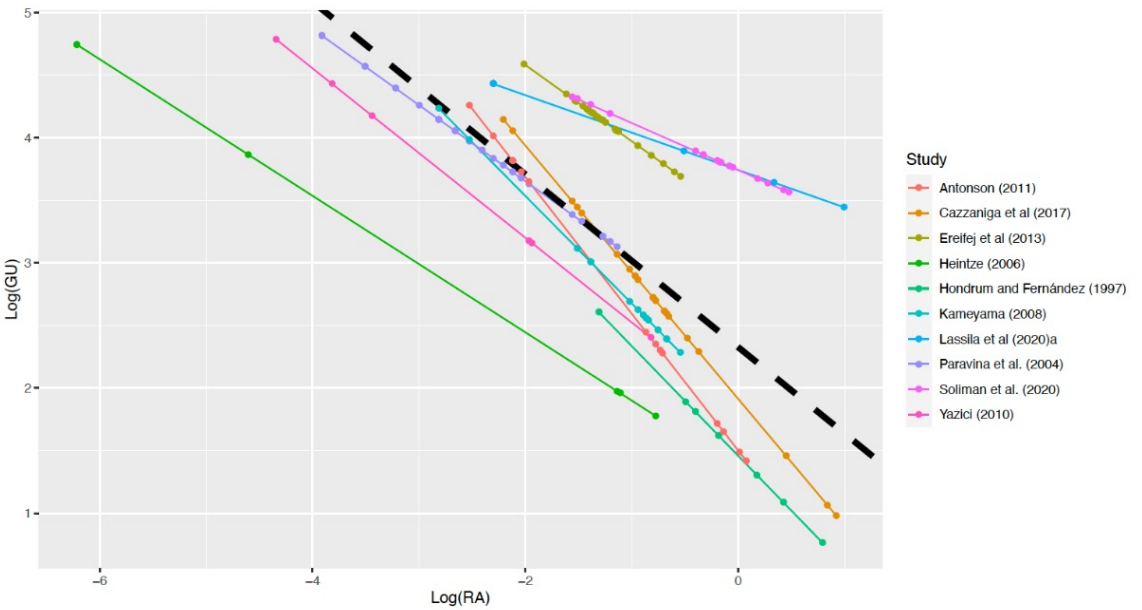

Figure 3. *The dashed black line represent the mean population effects—is the straight line with intercept and slope and . The other lines represent study specific-estimates indicating how the conditional mean responses from each study deviate from the population trend.*

values. Heintze and Zimmerly further pointed out that the exponential increase takes place between 0.3 and 0.1 μm, and that SG measurements can be done to distinguish correctly polished materials. Egilmez and others also found a clear relationship between SG and SR in the resin composites tested by them. These authors report an exponential growth below 0.1 μm.[51] SR and SG have, however, not only been linearly correlated by the primary studies included in this review but also by previous authors. It is important to point out that the strength of the correlation varies according to the surface properties and incident angle. This makes SG an anisotropic characteristic.[50]

Gloss is dependent upon the lightness of the samples, which highlights that the shade of the resin composite is relevant when gloss is to be measured. Filler particles are associated with bulk scattering. A resin composite with higher filler load allows more light to be reflected, and this results not only in better optical properties but also higher gloss values.[52] Nonetheless, most of the variations seen in SG, in polymers, is governed by their surface texture.[53] As mentioned before, no evidence of difference between microhybrids, nanofilled, or submicron resin composites has been found in SG and SR.[35]

Different shades of the same composite exhibit differences in their properties, one of them being related to their polymerization kinetics, affecting final rate and degree of conversion, which, in turn, affects physico-mechanical properties.[54,55] This may mean that they would respond differently to finishing and polishing procedures, as the softer surface produced by incomplete polymerization is more susceptible to scratches and abrasions; however, no studies included in this review explored this variable.[28,55] Surface properties are also influenced by chemical and physical wear of the composites, and laboratory simulations of clinical conditions should be considered. Only one study included in this paper standardized the press-on force with which the polishing systems were used and evaluated its influence on SG and SR.[30] These authors found that the force applied while polishing has significant influence on SG. In the study of Antonson and others, there is also reference to the pressure applied while polishing.[27] These authors conducted a preliminary study evaluating operators and their polishing perception on the pressure and its impact on calibration. Nevertheless, there was no investigation on the influence of the pressure applied.

The glossmeters used in the primary studies have different measuring areas, and this may introduce gloss measurement variations. The measurement area is especially important in uneven surfaces with anisotropic texture, as smaller measurement areas can lessen the variation arising from topographic changes.[56] There is no agreed threshold to acceptable or unacceptable gloss values, as previously pointed out. Cook and Thomas proposed a scale for the SG of polymers. These authors state that values above 60 GU, and in between 60 and 70 GU, are acceptable; between 70 and 80 GU

Table 6: *Risk of Bias Assessment of the Primary Studies Included in This Review, Using a Yes/No Scale*

Study	SSC	SR	CG	PC	AOA	BOA	CRO	O
Lassila and others[10]	N	N	Y	Y	Y	N	Y	Moderate
Soliman and others[24]	N	N	Y	Y	N	N	N	High
Lolita and others[19]	N	N	Y	N	Y	N	Y	High
Lopes and others[8]	N	N	Y	Y	Y	N	N	High
Cazzaniga and others[25]	N	Y	Y	N	Y	N	N	Moderate
Kamonkhatinkul and others[20]	N	Y	Y	N	Y	N	N	High
Ereifej and others[26]	N	N	Y	N	N	N	Y	High
Antonson and others[27]	N	Y	Y	N	Y	Y	Y	Low
Yazici and others[28]	N	Y	Y	N	Y	Y	Y	Low
Kameyama and others[29]	N	Y	Y	Y	Y	N	N	Moderate
Heintze and others[30]	N	N	Y	Y	Y	N	Y	Moderate
Paravina and others[31]	N	Y	Y	N	Y	N	Y	Moderate
Hondrum and Fernandez[23]	N	N	Y	N	Y	N	Y	High

Abbreviations: SSC, sample size calculation; SR, sample randomization; CG, control group; PC, polishing Compliant with Sequence, rpm, and Water Cooling; AOA, Appropriate Outcome Assessment; BOA, Blinding of the Outcome Assessment; CRO, Correct Reporting of the Outcomes; O, overall.

are good; and beyond that are excellent.[57] Considering the model approach undertaken in this study, it was possible to prove that values >55 GU have a probability close to 100% of correlating with SR values below the so-called SR threshold of 0.2 μm, which is currently known to be rather a range.[12] These findings support Cook and Thomas' scale.

Currently, the gold standard method of evaluating surface texture after finishing/polishing of restorative materials is by measuring SR, which can only be accomplished *in vitro*. By validating SG as a method for assessing whether restorations are properly polished, this method could potentially be used clinically. A standard reference value could thus be formulated, and clinicians would have a direct quantative and objective measure to assess surface finish of their restorations. The complexity of the tooth surface in regards to microstructure and curvature, together with drawbacks to standardization of measurement readings *in vivo,* should be considered.[1,58] Feasibility of clinical extrapolation warrants further research, as clinical studies measuring this outcome are nonexistent.[1]

Further studies should evaluate surfaces with rougher texture and evaluate gloss simultaneously, as few studies included samples with SRs above 1.2 μm. Such data will allow us to optimize the model proposed in this review.

As limitations to this review, it is important to consider variations in incident lighting. These should be taken into account when assessing SG and comparing study to study. Standardization of SG measurements in dentistry is required. Furthermore, R_a values are highly variable from technique to technique, in determining SR, and direct comparisons should be cautious.[59] Nonetheless, the majority of the included studies measured the samples using a profilometer, and only one study used atomic force microscopy (AFM) measurements.

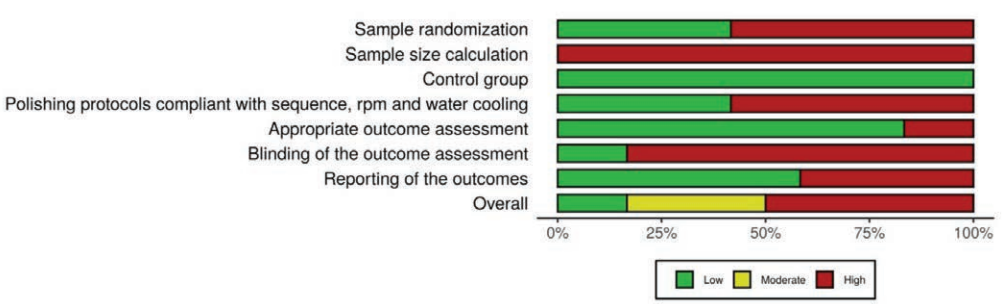

Figure 4. Weighted Plot Summary for Risk of Bias Assessment, Built Using the Robvis 2.0 Webtool.

Most studies included were of high or moderate risk of bias, which stresses the need for consistent methodological parameters in future laboratory studies. Compliance with sequence, rotations per minute (rpm), and water cooling of polishing/finishing systems is needed to standardize comparisons. Blinding of the outcome assessment by examiners and sample size calculations would also improve the internal validity of the studies. Selective reporting of outcomes was also an issue, with the correlations being determined in text but not shown in graphs and with no supporting model. It is important to understand that these differences may lead to overestimation or underestimation of results.[60,61]

CONCLUSIONS

Based on the results and limitations of the present review, the following can be concluded:

1. Evidence from laboratory studies that supports SG can be used to measure the effectiveness of finishing/polishing systems, as it is positively correlated with a decrease in SR.
2. A large between-study variability was seen, whereas within-study differences were more homogeneous. At low SR values, SG does not change at the same proportion as it did in higher SR. There is a decrease in the slope of the linear regression at higher SG values.
3. Based on the linear, mixed-effects model from the pooled results, the threshold for SR (0.2 μm) corresponds to >55 GU. This should be considered the acceptable reference threshold for well-polished samples. Thus, the gloss of polished surfaces is recommended to be above this threshold.

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Conflict of Interest

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

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Development and Assessment of Bioactive Coatings for the Prevention of Recurrent Caries Around Resin Composite Restorations

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Clinical Relevance

The application of bioactive surface coatings potentially contributes to the *in vitro* prevention of recurrent caries in enamel and dentin—a major cause of failure of resin composite restorations.

SUMMARY

Objective: To develop hydrophilic resin-based surface coatings containing bioactive agents (proanthocyanidins from *Vitis vinifera* and calcium silicate) and assess their protective role at the dentin and enamel margins of cervical restorations against demineralization under simulated conditions of high and low caries activity. Methods: Suboptimal resin composite restorations were placed on cervical cavity preparations on buccal and lingual surfaces of thirty-two molars after a contamination protocol. Groups were divided according to the resin-based coatings (n=8): resin without bioactive (C), resin containing 2% enriched *Vitis Vinifera* (VVE), and resin coat containing 10% calcium silicate (CaSi). The control group did not receive a resin (NC). To simulate a hydrolytic-enzymatic degradation, specimens were subjected to 2-month storage followed by incubation in esterase at 37°C for 8 days. Afterwards, recurrent caries was induced using a pH-proteolytic model on half of the specimens to simulate high caries activity, and the other half remained in simulated body fluid (SBF). Measurements of cross-section

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microhardness (KHN) and infiltration with rhodamine-B assessed the micropermeability (MP), the extent of demineralization (ED), and the demineralization area (DA). Data were analyzed using analysis of variance (ANOVA) and post-hoc tests (α=0.05). Results: VVE and CaSi presented higher cross-sectional KHN values for enamel and dentin (p<0.001). The bioactive coatings resulted in lower MP, ED, and DA compared to NC (p<0.005) in enamel and dentin. CaSi coating preserved the enamel from demineralization (p=0.160). Conclusion: The application of bioactive coatings represents a potential strategy to protect the enamel-dentin margins of resin restorations.

INTRODUCTION

Recurrent caries is the main reason for the replacement of adhesive restorations (up to 59%), despite the advancements in restorative techniques and materials.[1,2] Randomized clinical trials have shown that cervical resin composite restorations represent a clinical challenge due to deterioration of marginal integrity, regardless of the adhesion strategy.[3] As a result, these restorations fail due to the development of recurrent caries and bulk or marginal fracture.[3,4]

Resin-based restorations are technique sensitive and do not tolerate contamination and mishandling. Suboptimal adhesive sealing associated with known biologically driven degradative factors may further compromise the integrity of the restoration interface.[5,6] The biologically driven degradation of the adhesive interface involves two important mechanisms—the degradation of collagen fibrils by host matrix metalloproteinases (MMPs)[7] and the hydrolysis of dental resins by bacterial and salivary esterases.[8,9] Hence, the by-products of biodegradation at the adhesive interface promote bacterial growth and proteins associated with biofilm formation and acid production, contributing to caries formation and development.[10,11]

The use of surface coatings has been identified as a potential strategy to seal microcracks and microgaps at the adhesive interface.[12] The resin coating forms a barrier-like film layer that protects the restoration from physical, chemical, and biological aggressions[13] and might reduce dentin demineralization.[14] The addition of bioactive agents to these coatings may further protect and assist in the repair tooth-restoration interface, and provide additional protection to the surrounding enamel and dentin. Thus, the objective of this *in vitro* study was to develop hydrophilic resin-based surface coatings containing bioactives (proanthocyanidins from *Vitis vinifera* and calcium silicate) and assess their protective role at the dentin and enamel margins of cervical restorations under simulated conditions of high and low caries activity. The hypotheses were that under conditions of high and low caries activity the release of bioactive agents contributes to the prevention of recurrent caries development by maintaining mineral homeostasis around margins of cervical restorations. Also, the bioactive agents can decrease the micropermeability and demineralization of the enamel and dentin adjacent to the restorations.

METHODS AND MATERIALS

Synthesis of Bioactive Resin-based Coatings

A hydrophilic resin-based formulation was developed to release bioactive materials due to high degradability in the oral environment. The formulation of the experimental hydrophilic coating was obtained from pilot tests and previous formulations of an experimental neat resin.[15] Concentrations of 34.55 wt% 2.2-*bis* [4-(2-hydroxy-3-methacryloylpropoxy)]-phenylpropane (*Bis*-GMA), 15.08 wt% triethyleneglycol dimethacrylate (TEGDMA), 0.075 wt% camphorquinone (CQ), 0.3 wt% 3 ethyl dimethyl-4-aminobenzoate (EDMAB), 40 wt% 2-hydroxyethyl methacrylate (HEMA), and 10 wt% ethanol were used for the formulation of the experimental coating. The concentration of hydrophilic monomer (HEMA) was established to increase the hydrolytic degradation of the coating. Also, the effect of the addition of solvent (10% ethanol) and air evaporation (2 minutes before light curing) was assessed. This formulation was determined to allow the gradual release of bioactive agents to the tissues adjacent to the restoration.

The investigated bioactive compounds were: oligomeric proanthocyanidin-enriched (OPACs) extract from grape *Vitis Vinifera* seeds (VV_E)[16] and calcium silicate (CaSi).[17] Thus, hydrophilic resin coatings were formulated as follows: Coating-resin without bioactive; VV_E-coating containing 2% (w/w) VV_E; CaSi-coating containing 10% (w/w) calcium silicate.

Preparation of Resin Composite Restorations and Coating Treatment

Thirty-two sound human third molars were selected from a pool of extracted teeth stored at −20°C for no more than 6 months. Sections 4.0 mm above and below the cementoenamel junction (CEJ) were made using a diamond blade (Series 15LC Diamond Saw, Buehler, Lake Bluff, IL, USA). Cervical cavity preparations of 4.0 mm width × 3.0 mm length × 1.5 mm depth were prepared at the CEJ using a flat-end cylinder diamond bur (#557D, 1-mm diameter, medium grit, ISO 15223,

Brasseler USA Dental, Savannah, GA, USA) attached to a high-speed handpiece with water irrigation. Enamel and root dentin margins were prepared at a 90° angle to the tooth surface, and burs were replaced by new ones every five preparations. Cavity preparations were assigned to experimental groups (n=16) and further divided into subgroups (n=8) of either high- or low-activity condition.

A contamination protocol[18,19] was employed to create suboptimal adhesive interfaces, allowing better visualization of the effect of bioactive coatings in critical areas. Fresh saliva was collected from one of the investigators at the same time of day, 2 hours after food intake.[19] The restorative protocol consisted of etching with 32% phosphoric acid (Scotchbond Universal etchant, 3M Oral Care, St Paul, MN, USA) the enamel and dentin for 30 and 15 seconds, respectively, followed by water rinsing for 30 seconds and drying with an absorbent tissue. The collected saliva (0.1 mL) was applied to the dentin surface[18] using a microbrush for 20 seconds, followed by air drying for 5 seconds.[19] A layer of adhesive (Adper Single Bond Plus, 3M Oral Care) was actively applied on the contaminated surface, air dried to remove the excess of solvent, and light cured for 20 seconds (1200 mW/cm^2, Elipar, Deep Cure-S, 3M Oral Care). Resin composite (Filtek Supreme Ultra - shade A2B, 3M Oral Care) was inserted in three increments, and each layer was light cured for 20 seconds. All restorations were polished using a slow-speed handpiece with aluminum oxide disks (Soft-Lex Pop-On, 3M Oral Care) and then stored in distilled water at 37°C for 24 hours. Teeth were sectioned axially in the mesial-distal direction to separate the buccal and lingual restorations (Figure 1).

The specimens were coated with nail polish, except for the restorations and 2 mm around the restorations. Experimental resin coatings were applied up to 1 mm beyond the restoration margins and light cured for 20 seconds. An additional group had no coating application.

Aging Protocol

All specimens were subjected to 2-month storage in SBF (5 mM HEPES, 2.5 mM $CaCl_2$, 0.05 mM $ZnCl_2$, and 0.3 mM NaN_3) at 37°C[20]; replaced every 2 weeks. After SBF storage, an enzymatic-driven resin degradation protocol was carried out using 30 U/mL of porcine liver esterase (PLE, Sigma-Aldrich, St. Louis, MO, USA) in 0.2 M phosphate buffer (pH 7.2) at 37°C in a shaker for 8 days.[21] The esterase solution was renewed every 3 days.

High and Low Caries Activity Simulation

After the simulated aging protocol was completed, the specimens were divided into two subgroups: high and low activity. The restorations on the buccal surface were allocated to the high-activity group and those on the lingual surface to the low-activity condition. The high-activity group was exposed to periods of imbalance of de-remineralization associated with dentin-targeted proteolytic processes.

In demineralization processes, the progression of dentin loss increases, with the presence of collagen degrading proteases present in the oral cavity. Therefore, the protocol included a 16-day pH/proteolytic cycle of 7-hour immersion in demineralization buffer (50 mM acetate, 2.25 mM $CaCl_2 \cdot 2H_2O$, 1.35 mM KH_2PO_4, 130 mM KCl, pH 5.0), followed by 17-hour immersion in neutral buffer (20 mM HEPES, 2.25 mM $CaCl_2 \cdot 2H_2O$,

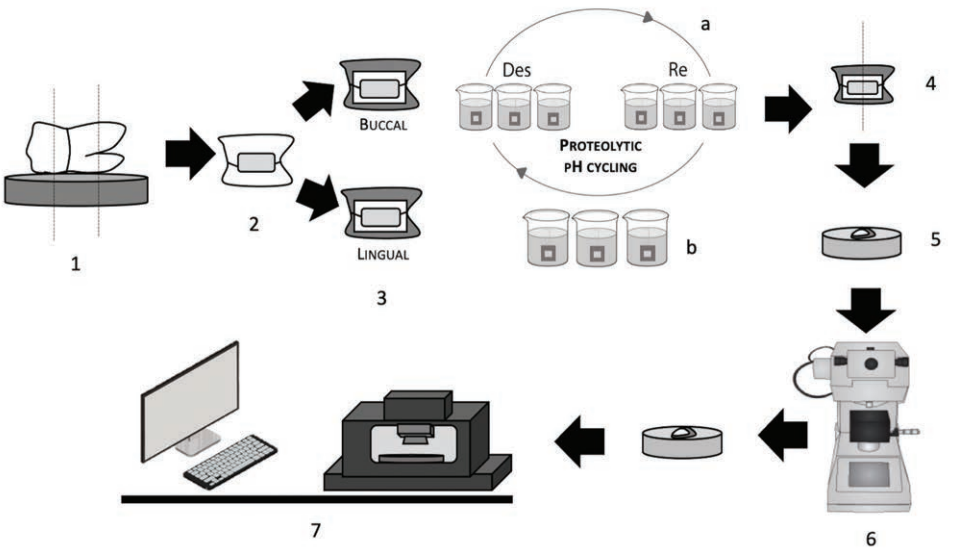

Figure 1. *Schematic of the experimental design. (1) Sections of the cervical portion of the tooth. (2) Cavity preparation with margins in enamel and dentin. (3) Surface blockage of areas away from the margin of restorations with nail polish. (a) Simulated high caries activity: buccal restorations follow pH-enzymatic/proteolytic cycles; (b) simulated low caries activity: lingual restorations were kept in simulated body fluid. (4) Restorations were sectioned in halves; (5) Specimens embedded in epoxy resin; (6) cross-sectional evaluation of microhardness (KHN), and (7) permeability at and around the margins of the restorations.*

1.35 mM KH_2PO_4, 130 mM KCl, pH 7.0) at 37°C.[22,23] Periods in demineralization buffer for 96 hours at 37°C,[22,23] were employed in order to guarantee lesion formation. For the biodegradation of the dentin matrix by exogenous protease, bacterial collagenase (*Clostridium histolyticum* 100 μg/mL; Sigma-Aldrich) was added to the neutral buffer.[22,23] All solutions were prepared daily. The specimens assigned to the low-activity group (lingual restorations, n=8) remained stored in SBF during the same period (remaining as control), without de-remineralization and dentin-targeted proteolytic challenges.

Assessment of Caries—Cross-section Microhardness (KHN)

The development of artificial recurrent caries was determined by the assessment of cross-section microhardness (KHN) measurements of enamel and dentin[22,24] (n=5). The buccal and lingual restorations were sectioned in half (IsoMet 1000, Buehler). The sections were embedded in epoxy resin and polished (EcoMet 3000, Buehler) sequentially using 600-, 800-, and 1200-grit silicon carbide paper. The KHN of enamel and dentin adjacent to the cervical restoration was measured using Knoop indentation (LM700AT hardness tester, Leco Corporation, Michigan, USA) at a 25 g load force for 15 seconds.[22] Indentations were performed at four depths from the outer surface of the tooth (enamel/dentin) (20, 40, 60, and 80 μm) at three distances from the restoration boundaries (20, 60, and 120 μm) (Figure 2A). Three measurements were performed at each depth, and the average was obtained for each distance.

Assessment of Interfacial Micro-permeability and Demineralization Around Restoration—Fluorescence Microscopy

A qualitative and quantitative evaluation of the enamel-dentin/resin margins was assessed by a permeability method using overnight incubation in rhodamine-B fluorescent dye solution (100 ml of 0.1-mM rhodamine B in phosphate buffer, pH 7.2).[25] After elapse time, specimens were rinsed in running water for 1 minute and dried with absorbent paper. Specimens were examined immediately under a fluorescence microscope (DMI 6000 B. Leica, Buffalo Grove, IL,

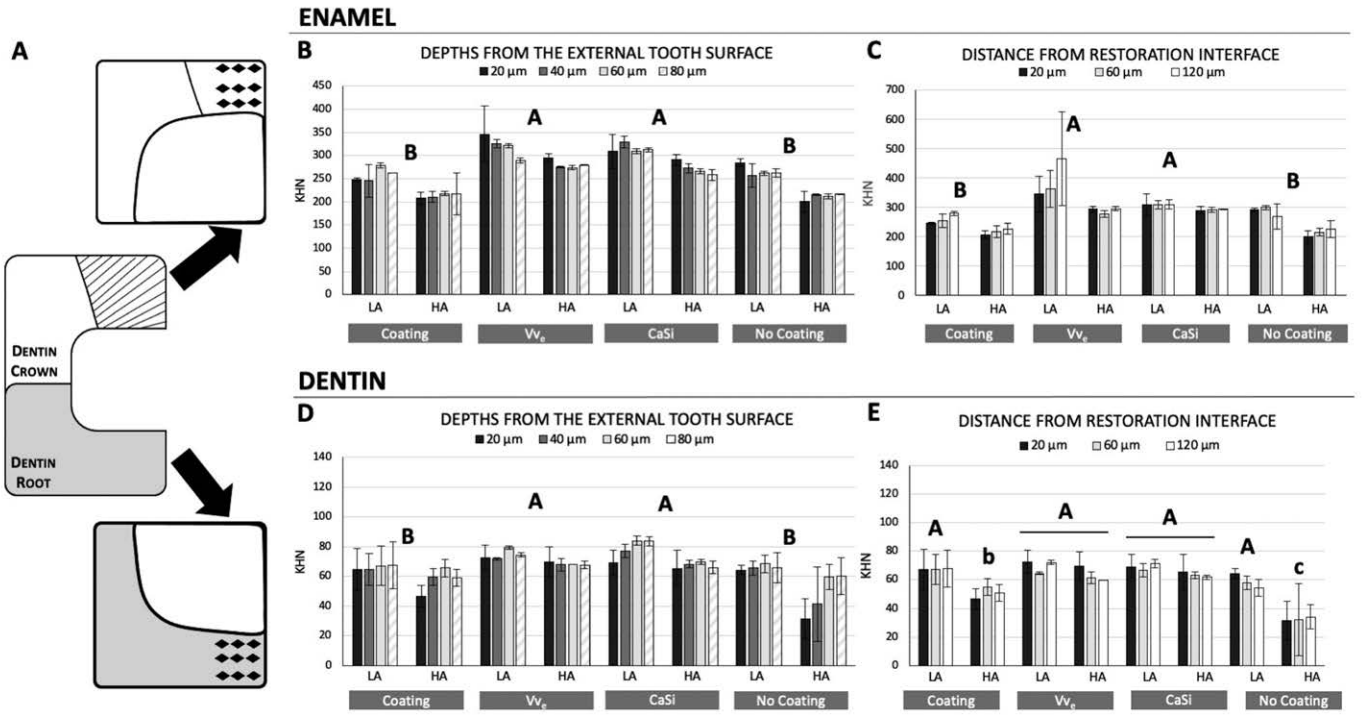

Figure 2. *Enamel and dentin microhardness (KHN). (A) Schematics of cross-sectional Knoop microhardness (KHN) measurements on enamel and dentin surrounding the restoration. KHN values of enamel (B) and dentin (D) treated with resin coatings as a function of depths (20, 40, 60, and 80 μm) from the outer surface to the enamel-dentin junction and inner dentin root. KHN values of enamel (C) and dentin (E) treated with resin coatings as a function of distance from the restoration interface (20, 60, and 120 μm). Different uppercase letters indicate differences between resin coatings (*p<0.05*). Different lowercase letters represent differences between low- (LA) and high-caries activity (HA) groups when the interaction effect between caries activity and resin coatings is significant (p<0.05). Bars represent lack of statistical difference between the low- and high-caries activity conditions (*p>0.05*).*

USA) using a connected digital camera (Hamamatsu, Skokie. IL, USA) and LAS AF software (Leica). The exposure time was 9.10 ms, gain: 30, FIM: 30%, IL-FID: 6 for red channel, and exposure of 25.28 ms, gain: 0, IL-FID: 9 using red emission and differential interference contrast (DIC) channels.

The margins of the restorations were identified in DIC images, and the measurements were done in fluorescence images.[25,26] After image acquisition of enamel and dentin, rhodamine infiltration was analyzed using the ImageJ software 1.48p (National Institutes of Health, Bethesda, MD, USA). Fluorescence emission intensity (FEI) was converted into numerical values, and values were calculated. Data analysis included quantification of interfacial micropermeability, the extent of demineralization depth from the restoration margin, and the demineralization area.

The interfacial micropermeability was measured using a parallel-line profile traced along with the adhesive interface, using ImageJ tools (Figure 3A).[26] The extent of demineralization from the restoration margin was measured by drawing a line parallel to the extent of rhodamine infiltration in the long axis of the tooth, within 300 μm from the margins of the restoration (Figure 3B). This evaluation verified if the coating protected the adhesive interface and the tissue adjacent to the restoration. Additionally, the area of infiltrated rhodamine-B was quantified to evaluate the influence of experimental coatings in the dentin and enamel adjacent to the restoration (Figure 3C).

Statistical Analysis

The assumption of homogenous data distribution was confirmed with Levene test and found not to be met for all the obtained results ($p<0.001$). The data were analyzed using three-way analysis of variance (ANOVA) for KHN and two-way ANOVA for FEI data, followed by Games-Howell post-hoc tests ($\alpha=0.05$) using SPSS software (SPSS V25, IBM Corp, Armonk, NY, USA).

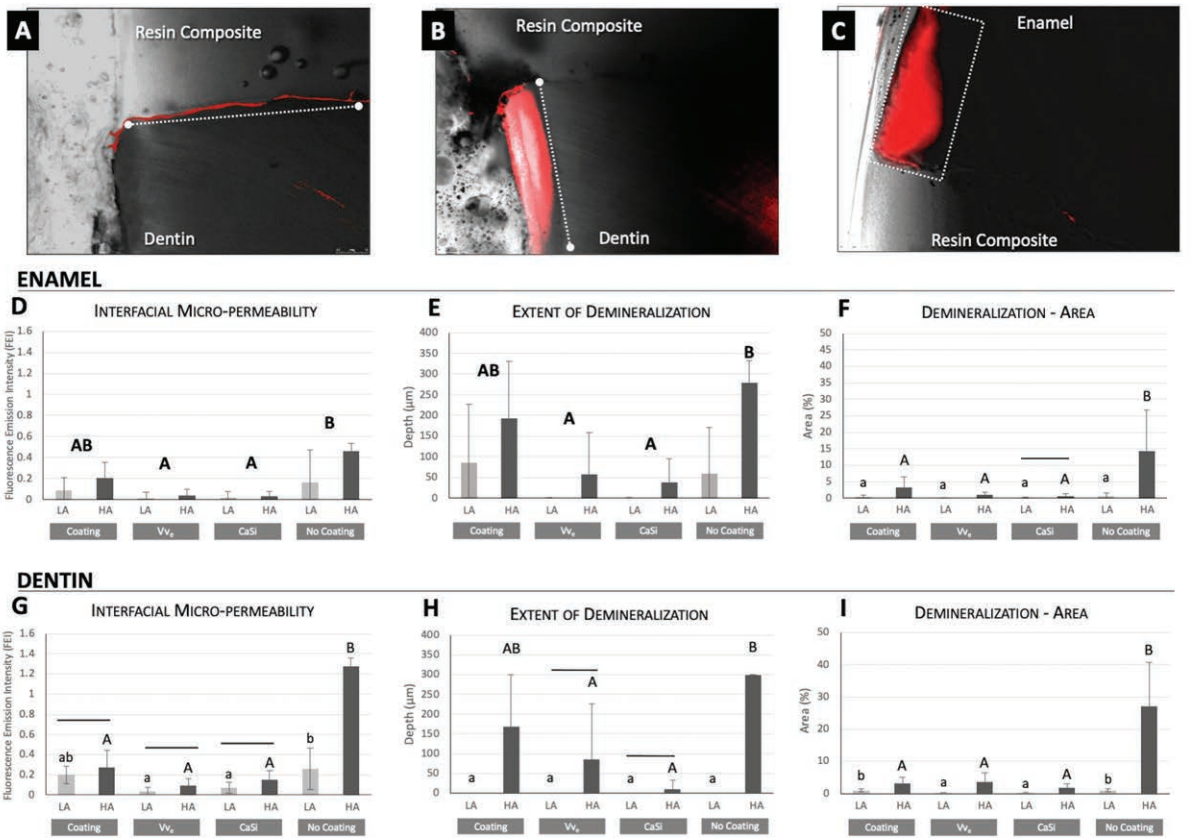

Figure 3. *Fluorescence microscopy representative images and results. Images depict measurements made on enamel and dentin margins to determine the (A) interfacial micropermeability, (B) extent of demineralization from the restoration margin, and (C) demineralization area. Results of the experimental groups after 2-month storage and pH-proteolytic cycling, under high- (HA) and low-caries activity (LA) conditions: (D,G) micropermeability at the tooth-restoration interface (%FEI), (E,H) the extent of demineralization from the restoration (mm), and (F,I) demineralization area (%). Different uppercase letters indicate differences between resin coatings* ($p<0.05$). *Different lowercase letters represent differences between low-caries activity treatment groups when the interaction effect between caries activity and resin coatings are present* ($p<0.05$). *Bars represent a lack of statistical difference between low- and high-caries activity* ($p>0.05$).

RESULTS

Degradation of Experimental Hydrophilic Resin-based Coatings

The experimental coating formulation containing 40% HEMA resulted in a significantly higher degradation as compared to the 6% HEMA concentration (p<0.001, Figure 4A). It was also observed that the mass loss (%) increased over 2 months (p=0.001). Knowing that the technique of applying ethanol-based resin can influence the degradation of the material, it was observed that the application of air evaporation of the solvent did not change the mass loss among groups; H40, with no ethanol; H40_10E (0), with 10% ethanol and immediate light-curing polymerization (no air evaporation); and H40_10E (2), 2-minutes air drying (p=0.070), even after 2 months (p=0.605, Figure 4B). Therefore, the selected base formulation was H40_10E (2): 2-minutes air drying to perform the hydrophilic resin coating.

Assessment of Enamel and Dentin Microhardness (KHN)

The cross-sectional KHN values of enamel and dentin are presented in Figure 2. Considering the KHN values of the depth from the external surface of the tooth (20, 40, 60, 80 mm), there was no significant interaction between all the variables tested (caries activity, depth, and experimental coatings) in enamel (p=0.561) and dentin (p=0.317). Overall, high activity resulted in lower values of cross-sectional KHN when compared to low activity, in both enamel and dentin (p<0.001). The groups with bioactive coatings (VVE and CaSi) resulted in significantly higher KHN for enamel and dentin (p<0.001) than coating and no-coating groups (Figure 2B,D). These KHN values refer to the depth of caries from the external surface of the tooth and thus can better explain the effects of the coatings that were applied on the restoration and the surrounding enamel/dentin.

The cross-sectional KHN values from restoration interface (20, 60, 120 mm) revealed the effect of coatings along with the internal wall of the restorations. No significant interaction was found between the caries activity groups, distance from restoration and experimental coatings for enamel (p=0.566), and dentin (p=0.910). In the enamel, VV_E and CaSi coatings presented significantly higher KHN values (p<0.001). Interestingly, there was a significant interaction between caries activity and experimental coating in the dentin (p=0.016). VV_E and CaSi coatings preserved the initial dentin KHN values even after high caries activity conditions, while coating and no-coating groups showed a reduction in KHN values when exposed to a high caries activity (Figure 2C,E).

Assessment of Interfacial Micropermeability and Demineralization Underlying Restoration

The results obtained from enamel margins are presented in Figure 3D-F. Results from the demineralization area in enamel showed significant interaction between the variables: experimental coating and caries activity (p<0.001). Although no significant differences were found among groups under a low-activity protocol (p=0.505), in high activity, the presence of coating showed less demineralization area when compared to the no-coating group (p<0.001). Only the CaSi bioactive coating preserved the enamel area in both the caries activity conditions (p=0.160) (Figure 3F). In enamel, no significant interaction between studied factors was

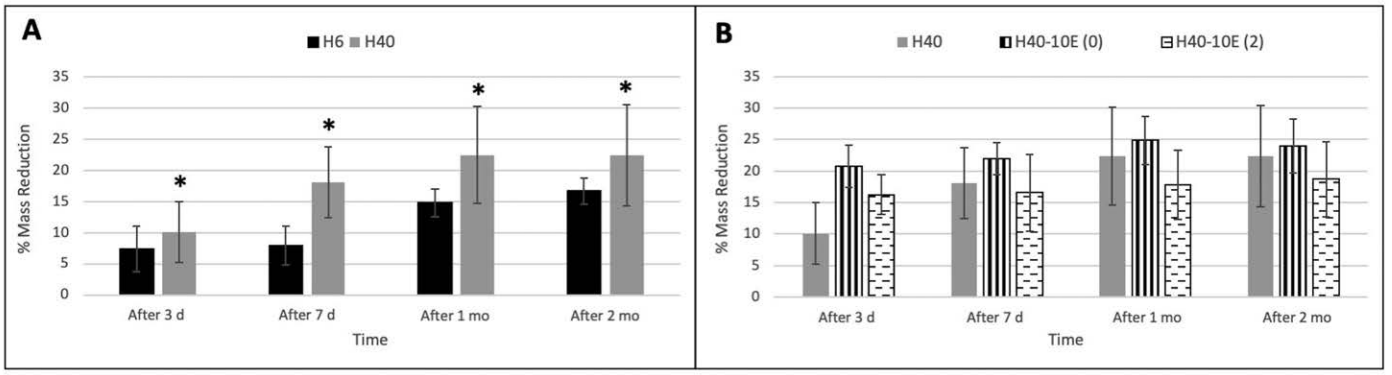

Figure 4. *Mass reduction of formulated resin coatings. (A) Initial results showed a higher mass reduction (%) for the most hydrophilic coating, containing 40% HEMA (H40) when compared to 6% HEMA (H6) (*p*<0.001), with a gradual increase over the 2 months (*p*=0.001). Asterisks (*) depict a statistical difference between the groups. (B) After the incorporation of ethanol (ETOH) and the use of solvent evaporation techniques, no difference was observed in the mass reduction (%) of developed coatings (*p*=0.070); H40-10E (0): coating with 40% HEMA + 10% ETOH (without solvent evaporation), H40-10E (2): 40% HEMA + 10% ETOH (with 2-minutes long solvent evaporation) or H40: adhesive with only 40% HEMA, even after 2 months (*p*=0.605).*

found for interfacial micropermeability (p=0.124) and the extent of demineralization (p=0.078). Bioactive coatings (VV_E and CaSi) reduced micropermeability (p<0.001) and extent of demineralization (p=0.001), when compared to the no-coating group (Figure 3,D-E).

In dentin, there was a significant interaction between the resin coatings and caries activity for interfacial micropermeability (p=0.003), the extent of demineralization (p<0.001), and area of demineralization (p<0.001). Different from the enamel, the application of bioactive coatings (VV_E and CaSi) had an effect under the low-activity condition, significantly reducing the demineralization area (p<0.001) and micropermeability (p=0.006), when compared to the no-coating group. In the high-activity condition, all coatings (Coating, VV_E, and CaSi) significantly decreased interfacial micropermeability (p=0.001) and dentin demineralization area (p<0.001) (Figure 3G,I). All the coatings prevented an increase of interfacial micropermeability when exposed to high-activity conditions, different from the no-coating group (p=0.02). Under high-activity conditions, less extension of demineralization was found in groups containing bioactive coatings (VV_E and CaSi) than in the no-coating group (p<0.001) (Figure 3H). However, none of the resin coatings was able to fully prevent the formation of recurrent caries.

DISCUSSION

Bioactive coatings with the potential to modify and protect the dental substrates may represent an effective strategy to prevent interfacial micropermeability and recurrent caries development around restorations. In this study, bioactive coatings assisted with preserving the KHN of the dentin and enamel surrounding the restoration. Hence, bioactive resin coatings contributed to the stability of the dentin and enamel without significant demineralization and an increase of interfacial micropermeability of suboptimal resin-based restorations. Therefore, the study hypotheses were confirmed.

The use of surface coatings has been shown to prevent tooth demineralization[14] and provide a seal of microcracks and microgaps in the resin.[12] Hence, the action of resin-based physical barrier[27] against root caries development is conditional to the wear resistance of the coating agent.[14] Also, greater exposure of the teeth to demineralization and by-products accelerates the coating's organic and inorganic degradation. Herein, an *in vitro* pH cycling model was designed to simulate high-activity conditions and reproduce the dynamic variations in mineral saturation and pH associated with the natural caries process.[28] Enzymatic degradation that occurs due to the presence of saliva esterases, microorganisms,[21] and proteolytic degradation[29] were also simulated under de-remineralization cycles.[22,23] As observed in this study, the enzymatic and proteolytic challenge induced a more significant dentin demineralization on uncoated or nonbioactive coating groups (Figure 3).

The effectiveness of the bioactive coating sustaining mineral homeostasis was assessed by detection of micropermeability. The penetration of a fluorescent dye into porosities of demineralized enamel/dentin enables quantitative analysis under fluorescence microscopy.[30] Higher micropermeability and demineralization values were observed under high-activity conditions compared to low activity (Figure 3). Also, the KHN results confirmed changes in the mineral gain or loss among the experimental conditions. A suboptimal adhesive interface[18,19] was utilized to simulate critical clinical challenges. Given the conditions evaluated in this study, the use of bioactive coatings (VV_E and CaSi) enhanced the protection of surface coatings on enamel and dentin surrounding restorations. Higher KHN values and lower micropermeability at the enamel adjacent to the restoration were observed with bioactive coatings, regardless of the bioactive agent (Figures 2 and 3).

Proanthocyanidins (PACs) are plant-derived dentin biomodification agents able to stabilize the organic matrix, improve mechanical properties,[31,32] and promote dentin remineralization.[22] Specifically, oligomeric PACs elicit greater bioactivity.[25] PACs' role on mineral homeostasis was observed earlier under a root dentin remineralization *in vitro* model.[33] Also, PACs from an enriched fraction of grape seed extract, when incorporated into adhesive systems, inhibited recurrent caries and contributed to the protection of the dentin-resin interface under a microbial model.[25] VV_E coating resulted in high KHN when compared to uncoated or nonbioactive surfaces (Figure 2). Also, reduced micropermeability and demineralization were observed for VV_E group (Figure 3).

Selective PAC-rich extracts preserve the integrity of the dentin and induce collagen crosslinks in the dentin extracellular matrix.[16] Furthermore, PACs decrease collagen digestibility by inhibiting proteases such as endogenous metalloproteinases.[34] These factors may have contributed to the slow progression of demineralization in the root dentin/enamel (Figure 3). The VV_E increases crosslinking of the collagen network, which represents a contributing factor preventing acid diffusion and mineral loss. Additionally, the calcium-binding ability of certain PAC compounds may contribute to mineral deposition during the remineralization process.[33] Previous studies have shown

that this process is associated with mineral deposition on the most superficial portion of the lesion, forming insoluble complexes in the presence of a remineralizing solution (pH 7.4).[22] These insoluble complexes have been shown to remain visually stable at pH in the range of 2.0-7.0.[22, 25]

Calcium silicate (CaSi) based materials assist in the formation of apatite,[35,36] increase the resistance to demineralization of dentin[36,37]; and for these reasons, have been used in dentistry as an endodontic sealer and for vital pulp therapy.[38] The tricalcium silicate was incorporated in the hydrophilic coating, because it releases calcium and forms hydroxyapatite crystals adjacent to the material on hydration, dissolving slowly in SBF solution[39,40] or oral fluids.[37] Ionic exchange of Na^+ or K^+ with H^+ or H_3O^+ occurs when tricalcium silicate materials are in contact with the SBF solution. A silica hydrogel [$Si(OH)_4$] layer is formed from this ionic exchange, increasing the solution's pH value. As the pH increases (alkalinizing activity), the silica hydrogel portion from the bioactive silica-based materials dissolves, leading to the breakdown of Si-O-Si bonds and silanol group formation (Si-OH). Thus, these silanol groups condense to create a SiO_2-rich layer on the surface. Ca^{2+} and PO_4^{2-} ions that cross a SiO_2-rich layer form heterogeneous nucleation of the initial calcium phosphate.[41] Over time, this process continues and is believed to help close gaps within the material-tooth interface.[42]

Caustic erosion of the dentin is caused due to the high alkaline nature of the calcium silicate-based materials, which helps penetrate the dentinal tubules and aggregate into the dentin. During this process, hydration promotes the expansion of tricalcium silicate, resulting in a good sealing ability.[42] These factors may explain the high KHN values in the enamel and dentin around the restoration protected with CaSi resin coatings (Figure 2). Additionally, the CaSi coating preserved the enamel from demineralization and did not allow the increase of micropermeability and extent of demineralization in the dentin (Figure 3). Energy-dispersive X-ray spectroscopy (EDX) composition of depth profile and IR analysis confirm that reactive calcium silicate placed in close contact with demineralized tissue helps in the formation of apatite by remineralizing without phosphorus,[37] reaching several depths of the dentin.[36] The presence of long parallel tags with minimal signs of degradation, and additional deposition of calcium and silicate particles into the dentinal tubules was shown to preserve the hybrid layer's integrity (resin-dentin interface).[17] However, our study results did not show significant differences in micropermeability after using CaSi coating compared to other coatings (Figure 3).

Based on the findings of this *in vitro* study, the mechanical barrier of a resin-based coating is not enough to prevent demineralization around restorations. The low caries activity condition does not promote major changes in cross-sectional KHN and tooth demineralization (micropermeability, demineralization from the surface, and demineralization area). However, the simulation of high caries activity can better demonstrate the influence of bioactive coatings (VV_E and CaSi) in the enamel and dentin. The release of bioactive materials, such as CaSi and VV_E, from the resin coating is a strategy that may enhance the prevention of recurrent caries in dental tissues. These study findings spotlighted the importance of caries activity assessment to provide high-risk patients with additional preventive measures against recurrent caries development.

CONCLUSION

Hydrophilic resin-based coatings containing calcium silicate or proanthocyanidins are potential strategies to prevent the development of recurrent caries. Bioactive resin coatings increased KHN, decreased micropermeability, and demineralization in enamel and dentin adjacent to resin composite restorations.

Acknowledgments

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies. The use of extracted human teeth was approved by Institutional Review Board of the University of Illinois at Chicago (protocol # 2018-0226).

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. The authors alone are responsible for the content and writing of this paper.

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Use of Computerized Microtomography, Energy Dispersive Spectroscopy, Scanning Electron Microscopy, and Atomic Force Microscopy to Monitor Effects of Adding Calcium to Bleaching Gels

LC Mendonça • MLA Rodrigues • AA Bicalho • GR daSilva • PS Quagliatto • CJ Soares

Clinical Relevance

Bleaching teeth with hydrogen peroxide gels containing calcium does not prevent mineral loss at the enamel surface. However, the demineralized regions do not exhibit an increase in surface roughness.

SUMMARY

Objectives: The aim of this study was to evaluate the mineral content, expressed by calcium (Ca) and phosphate (P), in dental enamel exposed to bleaching agents using micro-computed tomography (micro-CT), scanning electron

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microscopy (SEM), energy dispersive spectroscopy (EDS), and atomic force microscopy (AFM).

Methods: Sixty bovine dental enamel specimens were randomly divided into three groups (n=20): HP35ca (bleached using 35% hydrogen peroxide with Ca); HP35wca (bleached using 35% hydrogen peroxide without Ca); and control (without bleaching). Five specimens from each group were used for SEM and EDS analyses, 10 specimens were used for AFM analysis, and the remaining five specimens were used for micro-CT analysis. The pH of the gels was measured using a pH meter. The EDS and micro-CT data were analyzed using one-way ANOVA and Pearson's correlation test. The AFM data were analyzed using one-way ANOVA (α=0.05).

Results: The weight percentages of Ca and P obtained using EDS were similar between the bleached and control groups. Small, superficial changes were observed by SEM in the HP35wca group. The HP35ca group showed similar patterns to the control group. AFM results showed no significant changes in the enamel roughness in any of the tested groups. No significant difference in the volume or depth of structural enamel loss was found between gels with and without Ca. No mineral loss was observed in the dentin substrate. The EDS and micro-CT analysis data exhibited a high correlation (p<0.001).

Conclusion: The addition of Ca to the bleaching gel had no beneficial effect on the bleached tooth enamel in terms of composition, mineral loss, and surface roughness. Micro-CT results exhibited a high correlation with the EDS results.

INTRODUCTION

Chromatic alterations in teeth compromise the esthetics of the smile, adversely affecting the social and emotional behavior of patients.[1] Bleaching procedures are the preferred method for treating tooth discoloration because they involve a simple and minimally invasive protocol.[1] Although there have been many studies on bleaching treatments, the performance of bleaching agents has not been demonstrated fully.[1]

Hydrogen peroxide is the most commonly used bleaching agent.[1] Different theories have been proposed to explain its bleaching mechanism, which involves the penetration of hydrogen peroxide and its decomposition into oxygen molecules capable of breaking down pigment macromolecules. However, studies have claimed that free radicals released by hydrogen peroxide are unstable and unspecific and react with the inorganic enamel matrix in addition to the pigmented organic molecules.[2-5] Hydrogen peroxide can diffuse through the tooth enamel and dentin, releasing free radicals that oxidize the chromophores of molecules.[6,7] These chromophores, rich in electrons that absorb specific wavelengths of visible light, break down when attacked by free radicals.[8,9] Free radicals attack the double bonds responsible for the color of chromophores, thus making the teeth appear lighter in color.[8,9] Another theory suggested that peroxide causes minor morphological changes in the enamel that increase its opacity due to the dispersion of light and hide the underlying dentin layer.[10-13] Whitening agents can also function by oxidizing the fluorescent components in dentin, such as dentin phosphoproteins. Hydrogen peroxide can whiten the dentin by oxidizing the aromatic amino acids in the dentin phosphoprotein.[14]

Several studies claim that bleaching is a completely safe procedure.[15-17] However, enamel demineralization upon bleaching can cause alterations in the tooth such as an increase in roughness, reduction in microhardness, and changes in the superficial micromorphology.[18-22] To prevent demineralization (especially the loss of calcium and phosphate ions) and the reduction of enamel hardness during tooth bleaching,[23] calcium and fluoride are added to the gel composition.[24] A significant increase in enamel permeability and roughness and a decrease in microhardness compared to the untreated control group have been reported after bleaching with 35% hydrogen peroxide gel with Ca or fluoride.[21] Bleaching with 10% carbamide peroxide showed that the enamel was susceptible to mineral loss during the whitening treatment, but this loss was minimized by the addition of F and Ca to the whitening agents.[24]

Different methods have been used to evaluate the changes that occur after tooth enamel bleaching, including quantitative tests to assess the changes in physical properties and mineral composition via biochemical measurements and qualitative evaluations using different imaging techniques.[19,22,25-28] However, micro-computed tomography is of particular interest to researchers because it can quantify the enamel loss at the surface as well as the subsurface.[29]

The clinical relevance of the undesirable effects of dental bleaching on tooth structures has yet to be addressed.[15-22] The methods used for analyzing enamel mineral loss after bleaching are important to confirm the effect of bleaching, obtain new information, and determine the association between different and complementary methods. Therefore, the aim of this study was to evaluate the mineral loss in dental enamel exposed to bleaching agents by micro-computed

tomography (micro-CT), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and atomic force microscopy (AFM). The following hypotheses were tested: (1) micro-CT can be used as an alternative method to determine the loss of enamel and dentin structures; (2) the presence of calcium in the bleaching gel can reduce enamel mineral loss and retain the surface roughness; and (3) the enamel mineral loss occurs primarily at the surface of the enamel structure.

METHODS AND MATERIALS

Preparation of Specimens — Bleaching Procedures

Incisor teeth of bovine animals of equal ages were extracted immediately after sacrificing the animals. The teeth were stored in distilled water at -10°C for a maximum of 30 days. Specimens of dimensions 5 mm × 5 mm × 4 mm (approximately 1.5 mm: enamel and 2.5 mm: dentin) were obtained from the central region of the buccal surface of each tooth using a water-cooled, low-speed diamond saw (Buehler Ltd, Lake Bluff, IL, USA). The specimens were randomly divided into three groups (N=20): HP35ca [bleached using 35% hydrogen peroxide with Ca ions (Whiteness HP Blue Calcium - FGM, Joinvile, Brazil)], HP35wca [bleached using 35% hydrogen peroxide without Ca ions (Whiteness HP Maxx - FGM, Joinvile, Brazil)], and control (without the application of a bleaching gel). Five specimens from each group were used for SEM and EDS analyses, 10 specimens for AFM analysis, and the remaining five specimens for micro-CT analysis. For the micro-CT and AFM analyses, the specimens from the bleached groups were analyzed before and after the bleaching procedures, while the specimens from the control group were analyzed before and after immersion in artificial saliva. The tooth enamel surfaces of the specimens were regularized using 600-, 1000-, 1200-, and 1500-grit abrasive papers (Arotec, Cotia, SP, Brazil) and polished with a polishing cloth and 6-, 3-, 1-, and 0.5-μm diamond pastes (Arotec) in a polishing machine (Arotec) to standardize the surface. The lateral and bottom surfaces were covered with nail polish to isolate the contact of the products only to the specimen buccal surface during the treatments (Rísque, SP Brazil). The HP35wca group was treated for two 40-min sessions, with a 7-day interval. The HP35ca group was treated for two 45-min sessions, with three applications per session, every 15 min; there was also a 7-day interval between sessions. All bleaching procedures were performed according to the manufacturer's instructions (Table 1). The specimens were rinsed with a distilled water spray after each session and then immersed in artificial saliva at 37°C until the next application of the whitening gel. After the last session for each group, the specimens were rinsed and stored in distilled water. The specimens were ultrasonicated in distilled water for 10 min before all tests. The debris layer produced during specimen preparation was not intentionally removed before analyses.

Micro-CT Analysis

Herein, micro-CT (a 3D imaging technique that utilizes X-rays to see inside a sample, slice by slice)

Table 1: *Product Composition, pH Values, and Manufacturer's Recommendations for Use*

Material	Group	Treatment	Composition	Batch Number	pH	Manufacturer
Whiteness HP Blue Calcium - HP 35%	HP35ca	2 sessions of 40 min, with an interval of 7 days	HP 35% (after mixing the phases), thickeners, inherent pigment, neutralizing agents, calcium gluconate, glycol and purified water	010319	8.3 (0.3)	FGM (Joinville, SC, Brazil)
Whiteness HP Maxx HP 35%	HP35wca	2 sessions of 45 min each (3 applications of 15 min), with an interval of 7 days	HP 35% (after mixing the phases), thickeners, mixture of dyes, glycol, inorganic filler and deionized water	060619	6.8 (0.1)	FGM (Joinville, SC, Brazil)
Specimen without contact with bleaching gel	Control	Storage in artificial saliva	1.5 mm Ca and 0.9 mm P in 0.1 mm Tris buffer solution	—	7.0 (0.1)	—

was conducted (Nrecom software version 1.6.10.1; DataViewer software version 1.5.1.2; CTAn, version 1.13; CTVol, version 2.0; SkyScan Bruker Belgium). In the reconstructed image, the internal structure of the sample was analyzed. The reconstructed images were then overlaid using the DataViewer software (version 1.5.1.2, SkyScan, Bruker, Belgium). Comprehensive 3D image analysis capable techniques (such as morphometry, densitometry, and segmentation) and advanced image processing methods allowed the quantification of mineral loss at the surface and subsurface.[30]

The bleached tooth analysis methodology was based on a previous study that conducted micro-CT to evaluate the cusp deformation produced by resin composite restorations. The images of the prepared tooth (reference) and the image of the restored tooth (target) were overlaid, generating a difference in the volume of the image (Diff). This Diff image represents the volume of cusp deformation caused by the polymerization contraction of the composite resin restoration with high resolution.[30] A similar protocol was used in the present study to determine the structural alterations produced by the bleaching process.

In this study, specimens (n = 5) were scanned using a high-resolution micro-CT instrument with a resolution of 0.35 mm (Bruker, Kontich, Belgium). The device was set to the following configuration: 100 kV and 100 mA, a 0.11 mm Cu filter, an image pixel size of 13 μm, a resolution of 1632 × 1092, and a 0.6° rotation pitch. Three image slices were generated over 1850 ms with 20 random movements, resulting in 1692 image slices. NRecon software (Bruker) was used to reconstruct the images by adjusting to the appropriate parameters for smoothing and beam-hardening artifact correction. The images of each specimen before and after treatment were overlaid using DataViewer software (Bruker) and the difference between them (Diff) was used to characterize the mineral loss in the entire sample (enamel and dentin) on a cubic millimeter scale. These differences were measured by 3D morphometric analysis using CTAn software (Bruker) that also allowed the measurement of mineral loss in millimeters via 2D morphometric analysis. In a 2D longitudinal cross-section view of the specimen, a micrometer was used to measure the enamel and dentin mineral loss, which was represented by the difference in depth in the overlapping images. To guide the overlay process, cavities were made at the base of the specimen with spherical drills and subsequently filled with composite resin.

Prior to the study, pilot tests were performed to confirm the accuracy of the technique by superimposing two different scans of the same specimen, one without treatment and one when stored in distilled water, which showed no significant difference in the calculated tissue volume (Figure 1A). However, significant mineral loss was detected in the treated specimens stored in distilled water, as marked by the dark gray line at the specimen surface (Figure 1B).

SEM and EDS Analysis

The specimens were vacuum-plated with gold (Balzers, Berlin, Germany) and analyzed at a magnification of 20,000× (Zeiss, Jena, Germany) by SEM. The EDS software, model INCA X-act (Oxford, Abingdon-on-Thames, United Kingdom), was calibrated based on the information that the sample was covered with a 56-nm thick gold layer; therefore, the software was able to perform the adjusted calculation. The content of calcium (Ca) and phosphate (P) ions (wt%) on the enamel surface was measured using EDS (Oxford, Abingdon-on-Thames, United Kingdom). The Ca/P ratio was calculated for each specimen and compared to the stoichiometric ratio of hydroxyapatite (1.67). Five measurements were made per specimen in the area corresponding to the 20,000× magnification image. This image was consistently used for all measurements and was defined during the pilot experiment. EDS analyses were normalized to a 100% windowless detector that determines semi-quantitative evaluation

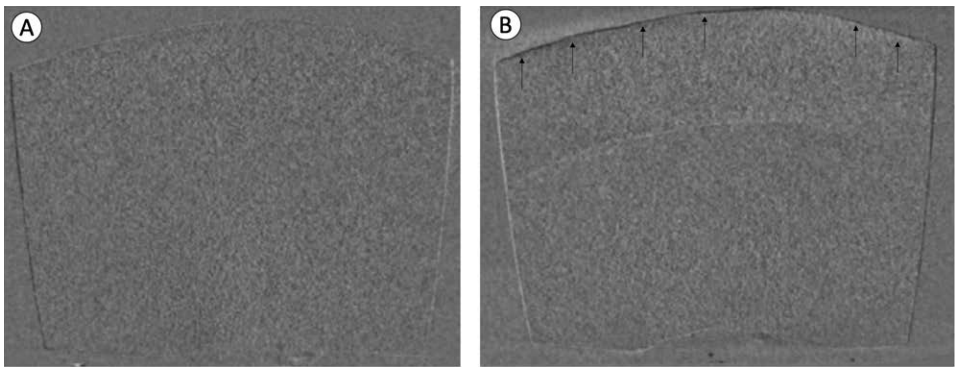

Figure 1. *(A) Representative image of the superposition of two scans of the same sample without any treatment and (B) representative image of the overlay of the previous scan image with the post-bleach image of the same sample.*

of lighter elements. The acquisition time was 30 s per measurement.

AFM Analysis

To determine the alteration of the surface roughness of the specimens, scans (in the positive X-axis direction) were performed on the specimens using an AFM machine in the dynamic force mode (Shimadzu, Chiyoda-ku, Tokyo). The probes (Tap190Al-G - Budget Sensors), which are designed to work at resonance frequencies between 160 and 220 kHz, performed the scans at the frequencies between 160 and 170 kHz without specimen distortion, damage, or removal from the surface. The scan speed (rate) was set to 0.5 Hz, collecting data from an area of 30 × 30 mm (resolution of 512 × 512 pixels) in approximately 18 min with a scan speed of 2 s per line. The equipment settings were as follows: operating point between 0.100 and 0.160, integral gain fixed at 1800, and proportional gain at 0. Using the Gwydion analysis software (version 2.57, open-source software for scanning probe microscopy data processing (http://gwyddin.net), 10 measurements (five vertical and five horizontal line scans) were extracted and the roughness of the specimen was determined.

Measurement of pH

The pH of the bleaching gels was measured with a pH meter (Adwa, Szeged, Hungary), which monitored the degree of acidity or alkalinity via an electrode coupled to a potentiometer (potential difference meter). The pH measurements were calibrated using a standard buffered potassium chloride solution. Three measurements were taken for each gel and an average was obtained. The pH electrode was calibrated with standard solutions before each measurement to ensure the sensitivity of the pH meter.

Statistical Analysis

After checking the data from micro-CT and EDS for normality (Shapiro-Wilks) and homogeneity (Levene), the volume and depth loss data for enamel and dentin obtained from micro-CT analysis and the volume losses of Ca and P measured using EDS were analyzed by one-way ANOVA. Surface roughness data measured by AFM were analyzed by one-way repeated measures ANOVA. All tests were performed using Sigma Plot (Systat Software Inc, Chicago, IL, USA) at a level of significance (α) of 0.05.

RESULTS

The means and standard deviations of the pH values of the HP35ca and HP35wca groups are shown in Table 1. The means and standard deviations of the loss of enamel (μm) in terms of depth and total volume loss (mm^3) are shown in Table 2. One-way ANOVA demonstrated no significant difference in the volume (p=0.001) or depth of enamel loss (p=0.001) of HP35wca and HP35ca groups. However, the bleached groups showed significant differences compared to the control group. No mineral loss was detected in the dentin substrate in any of the groups. The losses of the enamel structure in the HP35wca and HP35ca groups were located close to the surface. Micro-CT images showed similar volume losses for HP35wca and HP35ca (Figure 2).

The means and standard deviations of the Ca and P (wt%) compositions are listed in Table 3. The Ca (p=0.955) and P (p=0.393) contents analyzed by EDS were similar between the bleached and control groups. SEM images revealed superficial alterations in HP35wca, such as pores and depressions, and images obtained from the HP35ca group showed slight alterations (Figure 3).

The means and standard deviations of the surface roughness (Ra) are listed in Table 4. The Ra values were similar for all groups before (p=0.690) and after treatment (p=0.630). No significant variation was found before and after bleaching HP35ca (p=0.340), HP35wca (p=0.213), and control (p=0.412) groups. The AFM images showed no significant changes in any of the tested groups (Figure 4). The correlation coefficient for EDS data (Ca and P) and micro-CT data (depth and volume) is shown in Figure 5. Pearson's correlation exhibited high values between the Ca and P percentages measured by EDS and the depth and volume of mineral losses measured by micro-CT (p<0.001) for all combinations.

Table 2: *Means and Standard Deviations of Depth of Mineral Loss and Total Volume Between the Experimental Groups Obtained by Micro-CT, Calculated by One-Way ANOVA*[a]

Group	Depth of Loss (mm) (p<0.001)	Total Loss (mm^3) (p<0.001)
HP35ca	0.0326 (0.0008) B	0.4691 (0.2090) B
HP35wca	0.0338 (0.0008) B	0.4488 (0.2150) B
Artificial Saliva (control)	0.0064 (0.0008) A	0.0005 (0.0002) A

[a] *Uppercase letters establish relationships among columns. Different uppercase letters indicate statistically significant differences (p>0.05). The standard deviations are presented in parentheses.*

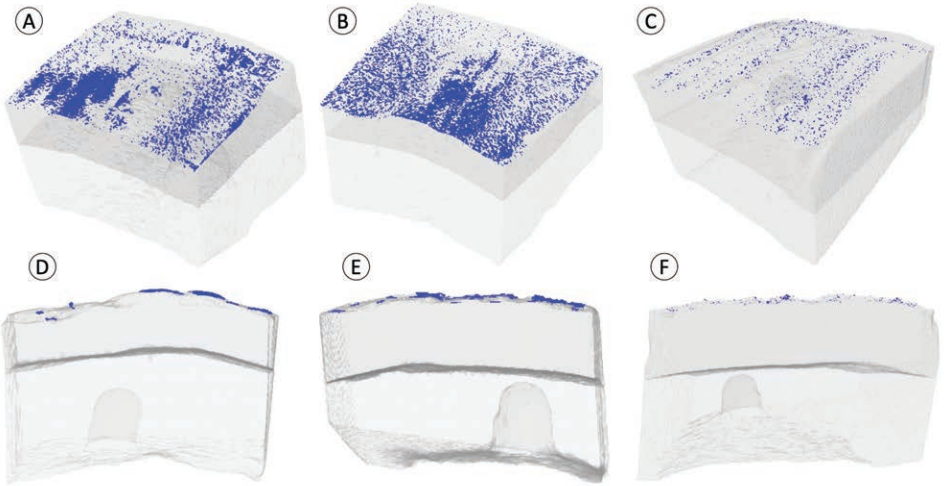

Figure 2. *Micro-CT images demonstrate similar volumes and depths of the loss of enamel structure in bleached groups. In the control group, only the difference related to the deviation of precision from the real measurement of the object is measured by the software. Representative image of depth loss for (A) HP35wca, (B) HP35ca, and (C) control. Representative image of enamel surface demonstrating volume loss for (D) HP35wca, (E) HP35ca, and (F) control.*

DISCUSSION

Micro-CT is a nondestructive method that allows the evaluation of the internal structure of the whitened enamel and can be used to evaluate the surface and subsurface enamel layers in 3D. The results of the study demonstrated that micro-CT can be recommended to assess the structural volume and depth loss of enamel and dentin tissues; therefore, the first hypothesis of our study was confirmed. Pilot tests performed prior to the study by overlaying two different scans of each specimen showed no significant differences when the images of specimens with no treatment were overlaid. In contrast, differences were observed between the treated specimens. In another study, the authors suggested that micro-CT was an adequate method to assess the mineral content of tooth enamel after the application of whitening gels.[31] However, the study tested only one group by applying 10% carbamide peroxide for two weeks, which caused enamel demineralization up to a depth of 50 micrometers below the enamel surface, and did not have a control group.[31] Although the effectiveness of the method in the analysis of the integrity of treated tissues has been proven, studies evaluating structural alterations after bleaching treatment using micro-CT are scarce.[32-35]

This study used overlapping images of the initial and final scans to show the surface alterations of enamel and the loss of enamel structure in terms of depth after using 35% hydrogen peroxide (HP) in-office bleaching gels, regardless of the calcium content in the gels. No mineral loss was observed in the dentin tissue using the resolution of the micro-CT analysis used in this study. New studies using nano-CT may detect mineral loss in the dentin; however, it is also important to consider the clinical relevance of minor mineral losses in the dentin caused by bleaching gels. Previous studies have shown similar morphological changes in enamel, and no changes in dentin have been reported after treatment with 37.5% HP and 35% HP. Ca and P decreased in the enamel and dentin with no significant differences between them or in relation to the untreated control specimens,[36] while another *in vitro* study with a 35% HP gel showed no evidence of deleterious effects of bleaching on enamel or dentin and suggested that studies reporting adverse effects on enamel and/or dentin actually reflected the pH of the formulation used.[37]

Table 3: *The Means and Standard Deviation of the Ca and P Values (wt%) and Ca/P Ratio in Enamel after Application of Bleaching Gels and in the Control Group Obtained by EDS*[a]

Group	Calcium (Ca) (wt%) (*p*=0.955)	Phosphate (P) (wt%) (*p*=0.393)	Ca/P Ratio (*p*=0.021)
HP35ca	36.5 (6.1) A	17.5 (2.4) A	2.1 (0.1) A
HP35wca	37.3 (4.8) A	19.3 (0.4) A	2.1 (0.2) A
Control	36.6 (2.3) A	17.5 (2.9) A	1.9 (0.1) A

[a] *The same uppercase letters indicate that there was no significant difference among the groups analyzed by one-way ANOVA (p>0.05). No significant difference among groups within columns is observed. The standard deviations are presented in parentheses.*

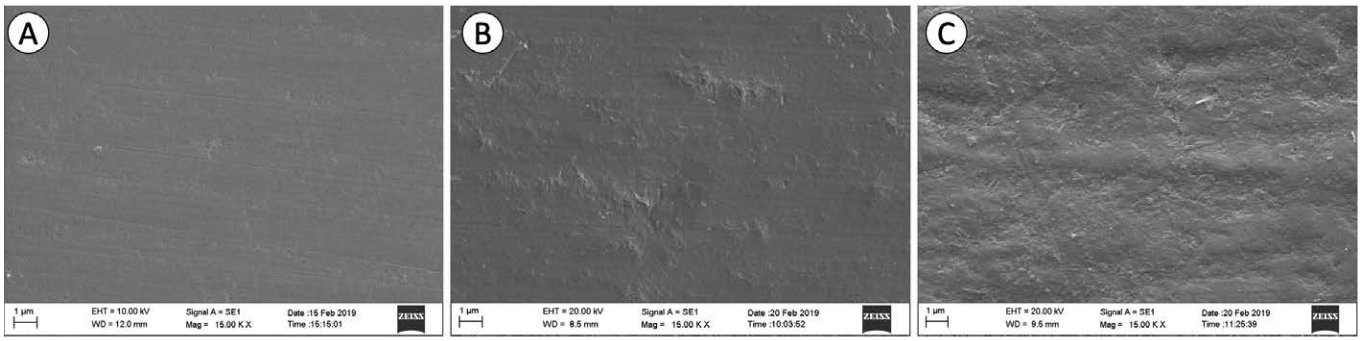

Figure 3. *Representative scanning electron microscopy images of the following tested enamels: (A) HP35wca application showing porosities and depressions; (B) HP35ca application showing slight surface alterations and areas with calcium deposition; and (C) no treatment (control group) showing no changes in the smooth polished surface.*

Ca is used in bleaching gels to achieve Ca ion supersaturation, preventing the dissolution of hydroxyapatite.[38] The optimal Ca concentration required during bleaching protocols is not well defined.[38] The addition of 0.5% calcium gluconate to a 35% HP gel was unable to prevent demineralization of the enamel[38;] therefore, this concentration was insufficient to supersaturate Ca ions relative to the enamel hydroxyapatite crystals.[38] A previous study found that bleaching enamel using a 35% HP gel containing sodium fluoride or calcium gluconate resulted in higher microhardness values than using gels without these compounds and that high concentrations of calcium gluconate in bleaching gels had a positive effect on enamel, but not at low concentrations in gels.[39]

Although studies have reported that the addition of Ca or fluoride to the 35% HP bleaching gel can reduce demineralization of the enamel surface,[40] it cannot fully prevent it or remineralize the subsurface enamel.[25] The increase in the permeability and roughness of the enamel surface and the decrease in the microhardness of the enamel are not prevented when Ca and F ions are added to 35% HP bleaching gels.[21] The results of the present study demonstrate the loss of the enamel structure in terms of volume by micro-CT in the studied groups; however, there was no significant difference in the results obtained by bleaching using HP gels with or without Ca. Therefore, the second hypothesis of this study was rejected because the presence of Ca of a certain concentration in the tested bleaching gel did not inhibit the mineral loss of the enamel structure. It is important to emphasize that the percentages of Ca and P on the enamel surface, which are the main constituents of hydroxyapatite crystals, were similar in both the bleached and control groups. Therefore, the 35% HP gel used for the 80-90 min in-office bleaching technique caused no significant change in the mineral composition of the tooth. Demineralization in some regions promoted the redistribution of minerals, as reported in previous studies,[34] which may explain the EDS results in our study. This result corroborates the results of those studies that used the same bleaching gels and determined from the EDS results that there was no statistically significant loss of Ca and P during treatment.[41]

Hydroxyapatite is a hydrated calcium phosphate from the mineral group of apatite, whose stoichiometric chemical formula is $Ca_{10}(PO_4)_6(OH)_2$ with a molar Ca/P ratio of 1.67.[42] The Ca/P ratios of the groups were calculated using the compositions of Ca and P obtained by microanalysis in EDS.[43] The calculated Ca/P ratio varied between 1.89 to 2.13 and showed no significant differences between the groups. Therefore, the presence or absence of calcium in the bleaching gel composition caused no significant alteration in the Ca/P molar ratio. In a previous study, where in a whitening treatment with 10% carbamide peroxide (CP) containing calcium (Ca) or amorphous calcium phosphate (ACP) was conducted, concluded that the

Table 4: *Means and Standard Deviations of Ra (nm) between the Experimental Groups Obtained by AFM — One-Way Repeated Measures ANOVA*[a]

Group	Initial Ra (nm) (p=0.690)	Final Ra (nm) (p=0.630)
HP35ca	3.7 (1.3) Aa	2.7 (0.8) Aa
HP35wca	3.4 (1.2) Aa	2.8 (1.1) Aa
Artificial saliva (control)	3.1 (0.4) Aa	2.7 (0.8) Aa

[a]*The same uppercase letters indicate that the Ra values were similar for all groups before and after treatment. The same lowercase letters indicate that no significant variation was found before and after bleaching the HP35ca (*p*=0.340), HP35wca (*p*=0.213), and control (*p*=0.412) groups. The standard deviations are presented in parentheses.*

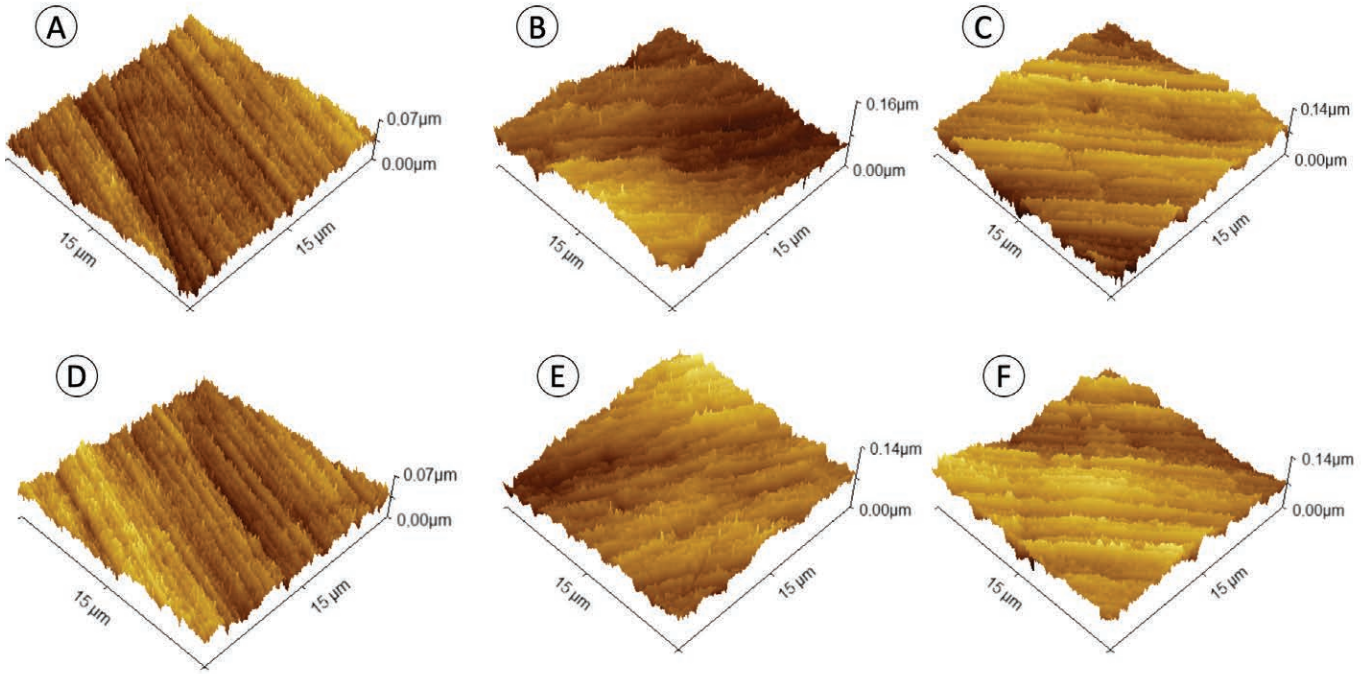

Figure 4. *Representative images of AFM of the following tested enamels: (A) and (D) representative images of the HP35wca group showing no significant changes on the enamel surface; (B) and (E) representative images of the HP35ca; and (C) and (F) without treatment (control group), showing no significant changes on the enamel surface.*

enamel microhardness decreased after the whitening process, regardless of the presence of Ca or ACP. However, no significant change in the enamel Ca/P ratio was detected, indicating that the bleaching gels have an erosive potential, causing enamel softening without promoting surface loss, irrespective of the presence of calcium or ACP ions.[44]

The concentration and duration of bleaching as well as the pH of bleaching agents can influence the mineral loss of bleached enamel.[22,45] An acidic pH causes changes in the mineral composition of the enamel structure,[4,46] contributing to enamel surface erosion.[33] No morphological or chemical alteration was found in the enamel surface in neutral or alkaline bleaching

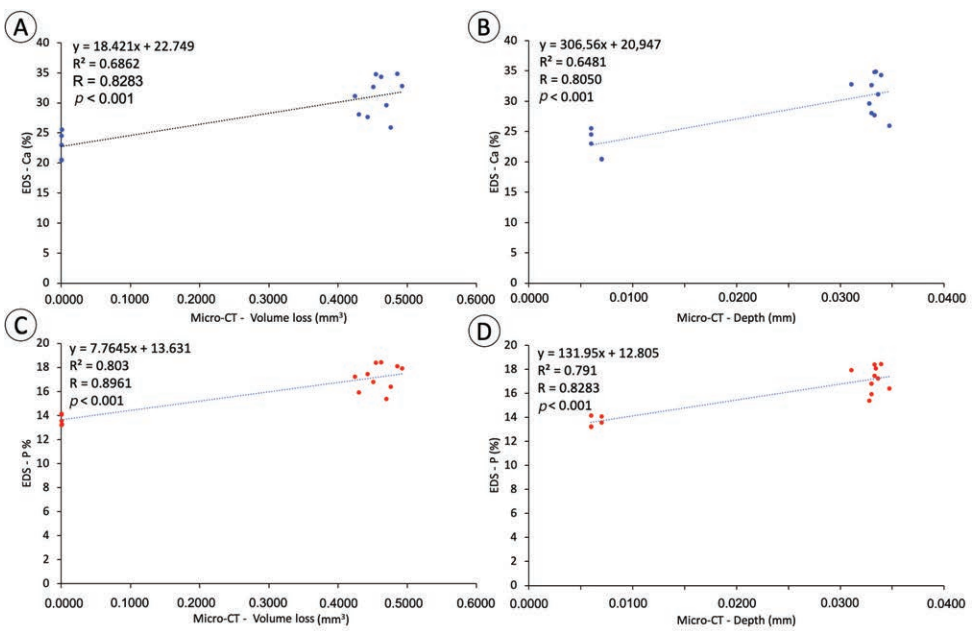

Figure 5. *Pearson's correlation between EDS and micro-CT data. Correlation between: (A) Ca percentage and volume of enamel tissue loss; (B) Ca percentage and depth of enamel tissue loss; (C) P percentage and volume of enamel tissue loss; and (D) P percentage and depth of enamel tissue loss; p < 0.001 for all tested correlations.*

solutions.[47] The tested HP35ca group had a basic pH (8.3), while the HP35wca group had an acidic pH (6.8), which may justify some of the changes in the enamel observed in the SEM images before and after bleaching. A representative HP35wca SEM image suggests the presence of pores and depressions on the enamel surface. The presence of Ca ions in the HP35ca group may have promoted crystal formation on the enamel surface.[48,49] However, the loss of enamel minerals was concentrated near the surface for groups treated with or without Ca, confirming the third hypothesis of our study. The application of silver nitrate to dental structures after bleaching with 35% HP gels in a previous study demonstrated moderate penetration of the enamel gel through the surface and subsurface prisms to a greater depth through the cracks and microcracks present in the enamel structure.[18] Transverse morphological observations with SEM in another study revealed morphological changes limited to a depth of less than 5 μm (0.005 mm) below the enamel surface with 30% HP gels for 30 or 180 min of immersion.[32] In this study, micro-CT showed significantly higher mineral loss for both bleached groups HP35wca group (0.033 mm) and HP35ca group (0.032 mm) than the control group (0.0064 mm).

The overlapping technique of micro-CT scans has proven to be a promising method to assess the loss of tooth structure caused by tooth whitening. A strong correlation was found between the EDS and micro-CT findings. The percentage of Ca and volume of enamel tissue loss (R=0.8283), the percentage of Ca and depth of enamel tissue loss (R=0.8050), the percentage of P and volume of enamel tissue loss (R=0.896), and the percentage of P and depth of enamel tissue loss (R=0.8283) are indicators of the efficiency of this method. Pilot tests also confirmed the accuracy of the technique by superimposing two different sweeps of the same specimen stored in distilled water, without treatment. There was no significant difference in the calculated tissue volume (Figure 1A), while significant mineral loss was detected in the treated samples and those stored in distilled water (Figure 1B).

In our study, AFM analysis showed no significant alteration in the surface roughness for all tested groups. Another study evaluated the effects of using in-office 35-40% HP bleaching gels with or without Ca or F on teeth and found that the 35% HP gel without Ca exhibited a slight increase in Ra, which was statistically different from the control.[50] On the other hand, another study that carried out bleaching using 20-45% CP gels and 9.5-38% HP gels reported no effect on the surface roughness.[51] A study carried out using 35% HP gels with and without calcium in the composition showed that the addition of calcium gluconate and the high and stable pH of the calcium-containing gel reduced tooth sensitivity in the study participants.[52] In our study, the presence of Ca in the bleaching gel showed no benefit; however, the study was designed using simulated artificial saliva containing Ca and P. For patients having a different saliva composition, the presence of Ca in the bleaching gel may prevent enamel demineralization.

One of the limitations of this study is the use of bovine teeth instead of human teeth. The majority of human teeth available for laboratory studies are extracted third molars. Because it is difficult to obtain human anterior incisors, the alternative bovine teeth were chosen. In this study, we opted to use bovine enamel due to its histological and structural similarity to human enamel.[53] Bovine enamel exhibits a reproducible surface, especially when its buccal surface is polished; hence, it can be safely used in a study that requires serial measurements.[54] Several related studies have used specimens of bovine teeth due to the difficulty in controlling the testing parameters with human teeth and the morphological variability of human teeth.[55] Other limitations are related to the use of gels with similar concentrations and classifications as well as different treatment protocols. The non-inclusion of other control groups indicated that saliva effects were not accounted for; therefore, the resolution limits of micro-CT could not be verified. This oversight did not consider the possible accumulation of debris resulting from the regularization and polishing of samples. In the present study, the control group, stored in saliva, did not have significantly different micro-CT, SEM, and AFM results. Therefore, the ions present in the formulation of the saliva did not interfere with the results. Artificial saliva was used to simulate the clinical environment and was replaced daily.[27] Studies *in situ* and *in vivo* have shown that the presence of saliva promotes remineralization on the enamel surface and does not make it porous.[56] Future studies are needed in order to test different resolutions of micro-CT; different devices with higher resolutions such as nano-CT can investigate different products with greater variability. A previous study evaluated whether there were significant long-term clinical benefits or side effects caused by the addition of ACP to CP16% whitening gel. The effects on tooth color, gingival health, and dentin hypersensitivity were evaluated after 90 and 180 days. After 180 days, the ACP group retained nearly 10% more of the original whitening treatment compared to that of the control. No other significant differences were found between groups. Tooth sensitivity, soft tissue health, and gingival health remained similar to baseline levels, proving the long-term safety of whitening treatment.[57]

Although we performed an *in vitro* evaluation using bovine teeth with artificial saliva, which does not accurately reproduce the clinical environment, these results have important clinical significance because they indicate that bleaching with a 35% HP gel can cause enamel demineralization regardless of the presence or absence of calcium in the gel. However, this demineralization did not change the surface roughness and the Ca and P levels on the surface of the treated enamel. Treatment with whitening gels is generally safe,[34] as long as it is performed by respecting the particularities of each patient and by a dentist. It is necessary to relativize the amount of mineral loss observed in this study with the clinical performance of bleaching procedures. Any adverse effects associated with use of the bleaching gel are temporary, easily controlled, and often disappear within minutes or hours of treatment.

CONCLUSIONS

Within the limitations of this study design, the following conclusions were drawn.

1. The micro-CT method was able to assess the loss of enamel structure in terms of volume and depth with a high correlation with EDS results.
2. The addition of Ca to the bleaching gel composition was not able to prevent enamel surface demineralization; that was minimal and superficial.
3. The enamel underwent mineral loss primarily near the surface regardless of whether bleaching gel, with or without Ca, was used; however, no alteration in surface roughness was observed.

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Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article

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