OPERATIVE DENTISTRY

May/Jun 2018
Volume 43
Number 3
225–336

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OPERATIVE DENTISTRY

Volume 43 / Number 3
May/June 2018

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Vol 43/No 3 May/June 2018 Pages 225–336 OPERATIVE DENTISTRY ISSN 0361-7734

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Clinical Technique/Case Report

Flared Roots Reinforced With Bulk-fill Flowable Composite — Case Report

R Braz • VA Mergulhão • LR Oliveira • MS Alves • CA Canto

Clinical Relevance

Flared roots are clinical situations in which the root dentinal walls are thin and consequently more prone to fracture. Clinicians must be aware of the treatment modalities and should consider bulk-fill flowable composite as a good alternative.

SUMMARY

This article presents a case report for the treatment of a patient with a flared root. The patient was treated with a bulk-fill flowable composite. This innovative approach seems to be efficient in reinforcing flared roots. The advantages and disadvantages of the technique are presented.

INTRODUCTION

Endodontically treated teeth may have weakened roots for several reasons, such as over instrumentation, internal resorption, immature development, tooth decay, previous restoration with an excessively large post, and fractures. The literature describes many techniques for restoring these teeth using different materials such as cast metal post-and-core and fiberglass posts associated with composite resin or cements. Although different treatment modalities have been described for the rehabilitation of flared roots, treatment is always a great challenge to clinicians.

Bulk-fill composites have an advantage in that they can be used in increments up to 4 mm thick.[1] This class of material shows low polymerization shrinkage stress and can be divided into groups with different rheological properties—low and high viscosity materials.[2] Bulk-fill composites contain a polymerization modulator that results in a slower modulus development, thereby reducing stress without decreasing the degree of conversion[2-4] and while keeping a good percentage of fillers; this makes their mechanical properties similar to those of conventional hybrid composites. Considering that the root canal is a cavity with a high configuration factor, we decided to use a bulk-fill flowable composite to reinforce flared roots and decrease the shrinkage curing stress. Therefore, the aim of the present case report is to demonstrate a technique that consisted of the reinforcement of a flared root with bulk-fill

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DOI: 10.2341/16-221-T

flowable composite, a glass fiber post, and a lithium disilicate glass ceramic crown (IPS e-max Press, Ivoclar Vivadent, São Paulo, Brazil).

CLINICAL CASE REPORT

A 50-year-old female patient showed up at the School of Dentistry (University of Pernambuco) reporting that she was dissatisfied with the appearance of the right lateral incisor. Clinical examination revealed that there was a temporary crown on the tooth, showing poor dental esthetics. Radiographic examination did not reveal any periapical lesions or periodontal damage, but showed a flared root caused by overinstrumentation during endodontic treatment. Since the root walls were thin (Figure 1), the need was evident to reinforce this root and improve the longevity of the tooth.

An initial periapical x-ray revealed that the working length had been set 1 mm short of the apex. A Gates Glidden drill size 4 was used to remove gutta-percha, leaving 4 mm of the material in the apical third and no gutta-percha on the lateral root walls, which was confirmed by another periapical x-ray.

Rehabilitation treatment consisted of root reinforcement with a bulk-fill flowable composite shade A1 (Filtek Bulk Fill Flowable Restorative, 3M ESPE, Sumaré, Brazil), a glass fiber post (White Post DC size 2, FGM, Joinville, Brazil), and the subsequent fabrication and cementation of a lithium disilicate glass ceramic crown (IPS e-max Press, Ivoclar Vivadent). Bulk-fill giomer and self-adhering flowable composite could have been used as an alternative to the bulk-fill flowable composite.

The temporary crown was removed, and the root dentin walls and remaining coronal structure were etched with 35% phosphoric acid etching gel (Scotchbond Universal Etchant, 3M ESPE) for 15 seconds, rinsed thoroughly with water, and dried with paper tips. Scotchbond Universal DCA was then mixed with Scotchbond Universal Adhesive (3M ESPE) in a ratio of 1:1 (drops) for five seconds immediately before application. Adhesive was applied to the entire tooth structure and rubbed in for 20 seconds (Figure 2). The excess was removed with paper tips and a gentle stream of air for about five seconds. The adhesive was then light-cured for 20 seconds using Optilight Max (Gnatus, Ribeirão Preto, Brazil). The light-curing unit had an intensity of 1200 mW/cm^2. Filtek Bulk Fill Flowable Restorative (3M ESPE) was inserted in the root canal to reinforce the flared root (Figure 3); then, a fiber post (White Post DC size 2), previously lubricated with glycerin gel, was

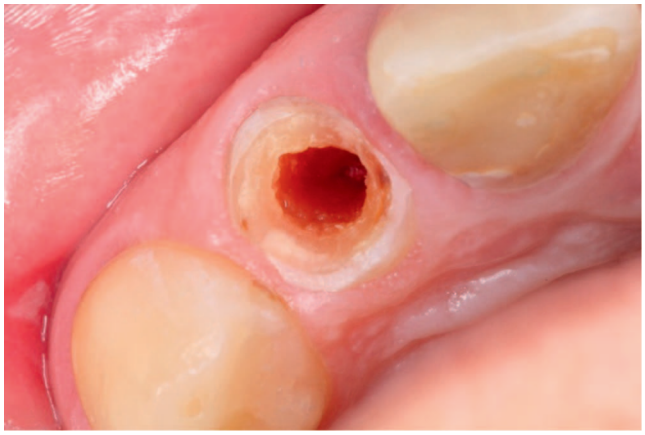


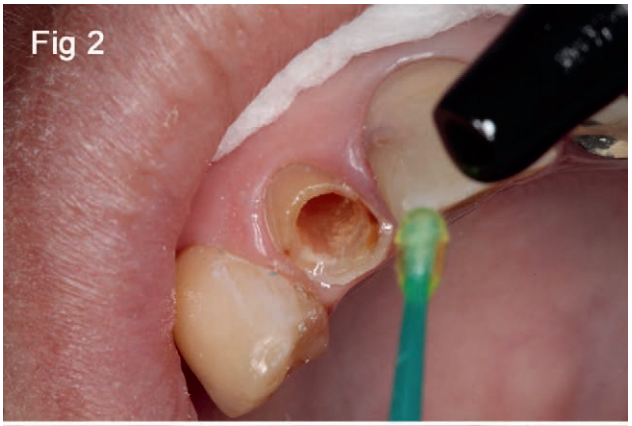
Figure 1. *Initial view of the flared root.*

inserted in the composite resin (Figure 4). Light activation was done on the top of the translucent post for 60 seconds. The fiber post was removed from the resinous root canal (Figure 5), and additional light activation was completed for 40 seconds, keeping the tip of the light-curing unit in contact with the entrance to the root canal.

The post was rinsed with water, dried with water-free and oil-free air, etched with Scotchbond Universal Etchant for 15 seconds, rinsed with water, and dried again. Since Scotchbond Universal Adhesive contains silane, there was no need to apply silane separately on the post. A disposable applicator was used to apply Scotchbond Universal Adhesive over the entire post surface to be luted, and it was light-cured for 20 seconds (Figure 6). The canal was copiously rinsed with water to remove the lubricant gel. The dual-cured resin cement RelyX Ultimate (3M ESPE) was applied on the post and in the canal, and the post was placed in the root reinforced with bulk-fill flowable composite (Figure 7). After removing the excess, the cement was polymerized through the translucent post for 40 seconds.

The coronal portion was built-up with Filtek Bulk Fill Flowable Restorative (Figure 8). After tooth preparation, a dual-mixed impression technique with a vinyl polysiloxane (Express, 3M ESPE) was used for the prepared lateral incisor; then, a lithium disilicate glass ceramic crown (IPS e-max Press) was fabricated.

Regarding the adhesive cementation, the inner surface of the lithium disilicate glass-ceramic restoration was etched with 10% hydrofluoric acid etching gel (FGM) for 20 seconds, rinsed thoroughly with water for 60 seconds, and dried with oil-free compressed air. Scotchbond Universal Adhesive was then applied to the entire surface to be luted



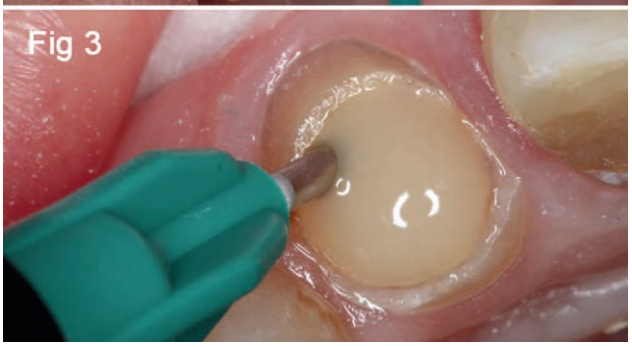


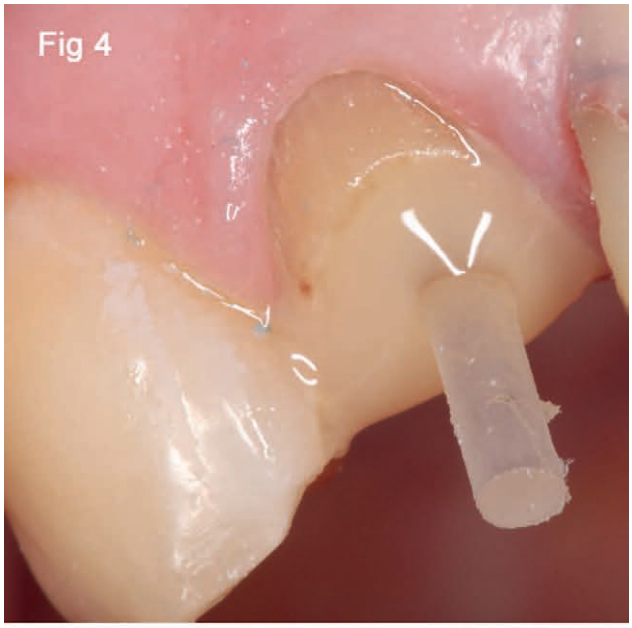
Figure 2. *Application of the adhesive to the root walls.*
Figure 3. *Insertion of the bulk-fill flowable composite.*

and allowed to react for 20 seconds. A gentle stream of air was directed over the liquid for about five seconds. The etch-and-rinse technique was used for the pretreatment of the coronal portion as described previously. To cement the metal-free crown, a small amount of RelyX Ultimate was dispensed on the inner surface of the restoration, which was seated to the prepared tooth, and the excess luting cement at the margin was removed. Two sides of the tooth were polymerized for 60 seconds (Figure 9). The materials used are listed in Table 1.

DISCUSSION

A flared root may show compromised longevity due to the thin remaining tooth structure. The treatment options vary considerably depending on the amount of remaining dentin for retaining the crown and the internal nature of the root structure. The most common options advocated in the literature include cast metal post-and-core, fiber post plus accessory posts, direct anatomic post (fiber post relined with composite resin), and indirect anatomic post.[5]

The dowel relining technique is a way of reducing the cement thickness in flared roots, decreasing the polymerization shrinkage stresses.[6,7] The importance of glass fiber–reinforced dowels relined with composite resin in flared roots is corroborated by other studies.[8,9] Amin and others[9] concluded that the group restored with glass fiber–reinforced dowels relined with composite resin in flared roots showed significantly higher fracture strength values



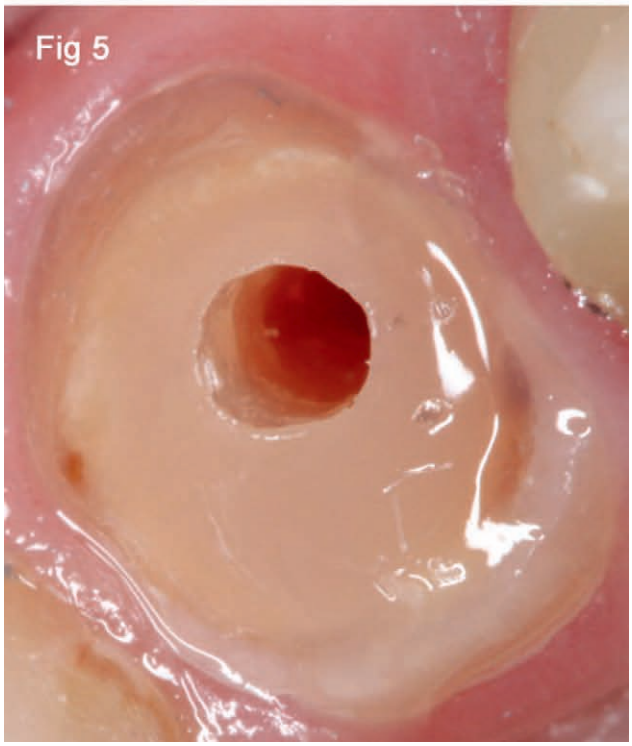


Figure 4. *Fiber post in the bulk-fill flowable composite.*
Figure 5. *Resinous root canal.*

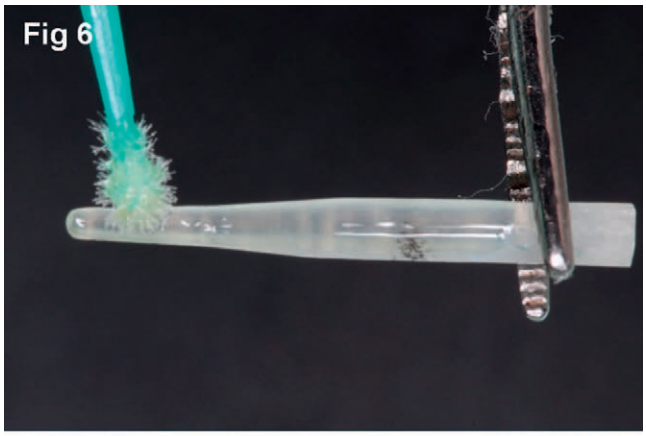
Table 1: Materials Used

Material	Manufacturer
35% phosphoric acid (etching gel)	3M ESPE (St Paul, MN, USA)
Scotchbond Universal Adhesive	3M ESPE (St Paul, MN, USA)
Optilight Max (light-emitting diode unit)	Gnatus (Ribeirão Preto, Brazil)
Filtek Bulk Fill Flow	3M ESPE (Sumaré, Brazil)
White Post DC (fiber post)	FGM (Joinville, Brazil)
RelyX Ultimate (resin cement)	3M ESPE (St Paul, MN, USA)
Express (vinyl polysiloxane)	3M ESPE (St Paul, MN, USA)
IPS e-max Press (Lithium disilicate-reinforced ceramic)	Ivoclar Vivadent (São Paulo, Brazil)
10% Hydrofluoric acid (porcelain etching gel)	FGM (Joinville, Brazil)

than the group restored with glass fiber–reinforced dowels and a thick layer of luting cement.

A case report[10] showed the rehabilitation of a flared root canal using a direct anatomic post (resin composite combined with a prefabricated glass-fiber post) associated with a metal-free ceramic restoration. The success of the treatment was noted after 3 years. Constâncio and others[11] presented a case report of the rehabilitation of anterior teeth with weakened roots. The authors used the direct anatomic post technique that consisted of inserting in the previously lubricated root canal a self-cure composite resin and then a fiber post plus accessory posts. The anatomic posts were cemented, and ceramic crowns were fabricated and placed on the prepared teeth. After six years, radiographic follow-up was conducted. The authors observed that the anatomic posts were in good condition and were biocompatible with the tooth and the restorative material; they concluded that the technique is low cost and saves time in the dental practice.

A finite element analysis study that simulated a maxillary incisor with excessive structure loss and flared root canals tested the effect of different



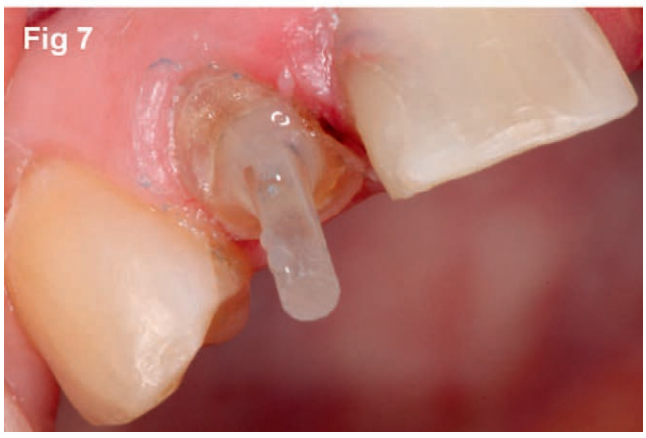
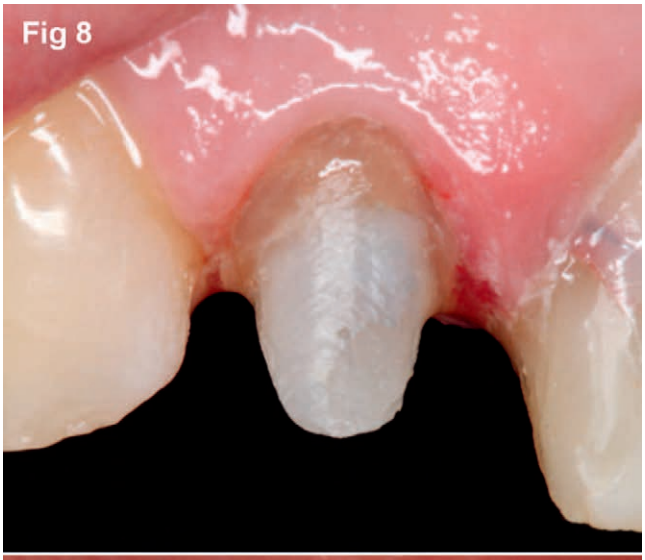


Figure 6. *Application of the adhesive on the post.*

Figure 7. *Fiber post cementation.*



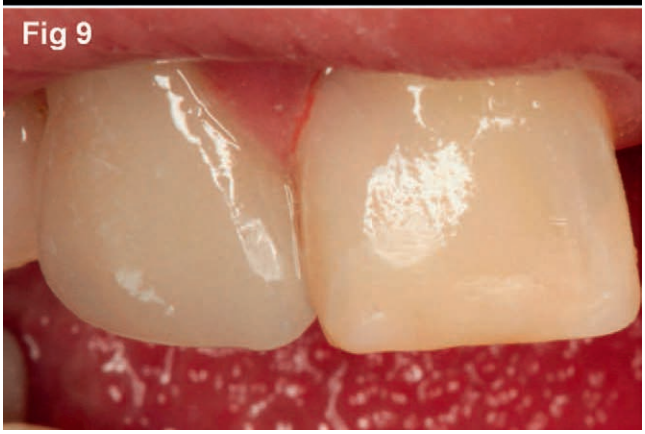


Figure 8. *Core build-up.*

Figure 9. *Final clinical result.*

restorative techniques on stress distribution in flared root canals. The researchers concluded that increasing the thickness of the root walls with composite resin produced less stress on the remaining root dentin structure but still showed higher total stress accumulation values at dentin compared with the anatomic post model. According to the results, anatomic posts maintained the stress inside the post body and generated less stress on the remaining root structure. The authors stated that anatomic posts may be safely used in roots with flared canals.[12]

Another study investigated whether fiber posts could lower the risk of post debonding and root fracture. Stress was analyzed in a three-dimensional finite element model of a premolar restored with a metallic or a fiber post. The fiber post produced lower stresses along the interface and higher stresses in the root. Therefore, fracture was less likely to occur in the root with the fiber post because its core and post fracture indices were higher.[13]

The first marketed light-curing bulk-fill resin composite (QuiXfil, Dentsply De Trey, Konstanz, Germany), a highly translucent material, was clinically tested for 4 years and showed satisfactory results.[14] Bulk-fill composite is a new class of material that can be inserted in the cavity in thick layers ranging from 4 mm to 5 mm due to their higher depth of curing.[15] In the present case report, we decided to reinforce the flared root canal with a bulk-fill flowable composite because it is a low-viscosity material that can be injected in the canal, saving time in the dental practice and avoiding bubble formation. The flow of Filtek Bulk Fill Flowable Restorative allows for easy adaptation in the root canal, which makes the technique faster than conventional methods. Another advantage is that this class of material shows low polymerization stress[16,17] because the composition includes modulators, which contributes to the decrease of bonding interface failures.

In the present case report, we used a bulk-fill flowable composite that combined triethylene glycol dimethacrylate (TEGDMA) and bisphenylglycidyl dimethacrylate. TEGDMA is a low-viscosity monomer, and this association of monomers promotes a fast propagation of the polymerization reaction by increasing the polymerization temperature. It facilitates the internal mobility of the monomers, which easily reach the reactive sites, thus increasing the degree of conversion (DC).[18] In addition, Filtek Bulk fill Flowable Restorative has about 10% to 20% of urethane dimethacrylate, a low molecular weight monomer incorporating an amino acid group which is responsible for chain transfer reactions that provide an alternative path for the continuation of polymerization.[19]

In addition to changes in the organic matrix composition of bulk-fill composites, two approaches have been adopted to increase conversion in depth.[20] The first is to increase translucency.[1] The second is to improve photoinitiator efficacy by incorporating additional photoinitiators, including trimethylbenzoyl-diphenylphosphine oxide (TPO) derivatives (Lucerin-TPO, Irgacure-819, GC, Tokyo, Japan) or benzoyl germanium compounds (Ivocerin, Ivoclar Vivadent), providing a synergistic effect to camphorquinone.[21] Li and others[22] compared the DC of bulk-fill composites with conventional that of composites and found that Filtek Bulk Fill Flowable Restorative (3M ESPE) the same composite used in the present case report, and Ever X Posterior (GC, Tokyo, Japan) showed the highest DC followed by SDR (Dentsply De Trey), Tetric EvoCeram Bulk Fill (Ivoclar Vivadent), and Herculite XRV Ultra (Kerr, Orange, CA). Bulk-fill composites have shown great light transmission compared with conventional composites.[23]

One of the questions that could arise regarding the technique used in this case report would be whether the bulk-fill flowable composite would have been properly light-cured in the apical region. It should be pointed out that the total root length was 11 mm. The post length was 6 mm in the root and 4 mm in the coronal portion. Considering that 4 mm of gutta-percha was left in the apical third and the working length was set 1 mm short of the apex, it must be concluded that no bulk-fill composite reached the apical root third. The composite remained in the cervical and middle root third.

A translucent post (White Post DC) was selected for the present case report. Morgan and others[24] assessed the luminous energy transmitted through different translucent fiber posts with a digital photometer. Blocks, consisting of posts in black resin, were submitted to sequential cuts at depths of 16 mm, 12 mm, 8 mm, and 4 mm. The results showed significant differences between different posts and depths. Comparing the posts, White Post DC exhibited the highest value of light transmission in the apical third (12-mm depth). In the middle third (8-mm depth), the group without the post (control) showed higher value of light transmission than the other groups. This suggests the importance of removing the post and light-curing the composite resin without the post as described in the present

case report. The results for the cervical third showed that the group without the post and the White Post DC group did not differ statistically.

The use of prolonged curing time and a decreased distance between the curing light and the composite to be polymerized produce a more effective curing.[25] This explains the importance of the additional light polymerization without the post, as presented in this case report. Durner and others[26] as well as Frauscher and Ilie[27] concluded that proper polymerization time (20 seconds and 40 seconds, respectively) with moderate irradiation (about 1000 mW/cm^2) is essential to ensure that the composite resin is properly polymerized and that a prolonged polymerization time (more than 40 seconds) resulted in a significant increase in the DC.

We carried out a laboratory study with extracted bovine teeth in order to evaluate the DC of bulk-fill composites (SDR and Filtek Bulk Fill Flowable Restorative) using Fourier transform infrared analysis under the same conditions of the technique presented. Four depths were tested: 4 mm, 6 mm, 9 mm, and 11 mm. There was no statistically significant difference at depths of 4 mm, 6 mm, and 9 mm. At the 11-mm depth there was a reduction in the DC for both composites. This demonstrates that the White Post DC has the ability to transmit light, without great dispersion, up to a depth of 9 mm. As the length of the post within the root in the present case report was 6 mm, it can be concluded that there was adequate light transmission by the post. This finding is consistent with the study by Taneja and others,[28] which demonstrated that when using translucent fiber posts there was insufficient light emission only in the apical third.

Seyam and Mobarak[29] evaluated the strengthening effect of resin composite cured by a modified layering technique for teeth with flared root canal. The materials used to reinforce the flared root canals were flowable resin composite, self-adhering flowable resin composite, dual-cure cement, and a translucent fiber post. A light-curing unit (800 mW/cm^2) was used that, according to the manufacturer, has the ability to keep good polymerization up to 7 mm depth. The degree of cure for each tested material was indirectly measured using microhardness at different root levels (cervical, middle, and apical), and fracture load results showed that there was no statistically significant difference from non-weakened teeth for either flowable composite. For each tested material, no significant difference was detected for microhardness values at different root levels.

While the present case report does not identify any disadvantage to using bulk-fill flowable composite to reinforce flared root canals, we suggest that in long flared roots, where polymerization is necessary in the apical third, dual-curing bulk-fill flowable composites need to be developed. Proper diagnosis and good treatment planning are essential to obtaining a good result for rehabilitation of patients with flared root canals. The technique presented in this article is innovative as bulk-fill flowable composites have not been tested for the purpose of reinforcing flared root canals. For this reason, more *in vitro* and *in vivo* studies are needed.

Conflict of Interest

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

(Accepted 22 July 2017)

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Clinical Research

Effectiveness of and Dental Sensitivity to At-home Bleaching With 4% and 10% Hydrogen Peroxide: A Randomized, Triple-blind Clinical Trial

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Clinical Relevance

At-home dental bleaching with 4% hydrogen peroxide is as effective as with 10% hydrogen peroxide, but it produces a lower risk for and less intense tooth sensitivity.

SUMMARY

Objectives: To evaluate the risk for and intensity of tooth sensitivity and color change of at-home dental bleaching with 4% and 10% hydrogen peroxide (HP).

Methods: For this study, 78 patients were selected according to the inclusion and exclusion criteria and randomized into two groups: HP 4 (White Class 4%, FGM) and HP 10 (White Class 10%, FGM). In both groups, the at-home bleaching was performed for a period of 30 minutes twice a day for two weeks. The color was assessed by Vita Classical, Vita Bleachedguide 3D-MASTER and spectrophotometer Vita Easyshade (Vita Zahnfabrik) at baseline, during bleaching (first and second weeks) and after bleaching (one month). Patients recorded their tooth sensitivity using a numeric rating scale (0-4) and visual analog scale (0-10). Data from color change (DeltaE data) was submitted to two-way analysis of variance. The color change data in Delta SGU from the two shade guide units were compared with the Mann Whitney test. The risk of tooth sensitivity was evaluated by χ^2 test and the intensity of tooth sensitivity from both scales was evaluated by a Mann-Whitney test (α=0.05).

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DOI: 10.2341/16-260-C

Results: The absolute risk and intensity of tooth sensitivity was higher in the group that used HP 10 than the one that used HP 4. Data from change in the number of shade guide units and color variation after one month of bleaching for both groups showed significant whitening, with no difference between groups.

Conclusions: At-home bleaching is effective with 4% and 10% HP concentrations, but 10% HP increased the absolute risk and intensity of tooth sensitivity during at-home bleaching.

INTRODUCTION

Dental bleaching is a popular procedure for treatment of discolored teeth.[1-4] Among the dentist-supervised techniques, this procedure can be performed at home or in the office. At-home dentist-supervised bleaching with custom trays is more popular than the in-office techniques due to some widely known advantages: lower risk and intensity of tooth sensitivity[5,6]; fewer required visits to the dental office; and lower cost to achieve the same whitening results as those produced with agents of higher concentration used in the in-office bleaching protocol.[7,8]

Haywood and Heymann[1] first described the at-home bleaching technique in 1989. The active 10% carbamide peroxide (CP) ingredient was delivered in a custom bleaching tray and worn overnight by the patient for two to three weeks. This bleaching modality has shown satisfactory clinical results.[7-13] However, since its introduction, the original technique has undergone some modifications. Changes in tray material, tray design, active ingredient concentrations, time of custom-tray use, and type of active ingredients are examples of these modifications.[14,15]

Some of these changes, such as the increase in the active CP gel concentration and the introduction of low hydrogen peroxide (HP)–based products for at-home vital bleaching, were envisioned to shorten the clinical time required to reach satisfactory color changes.[11,12,16,17] Different from CP gels, HP-based products undergo a faster degradation rate[18-21] and therefore should be used for shorter periods of time. Whereas a 10% CP gel contains 64% of active HP after one hour,[18] a 3% HP at-home bleaching agent contains only 32% of active HP after one hour.[20] This can be seen as advantageous for those patients who do not want to wear the bleaching tray for prolonged periods of time.

Currently, there are various concentrations of CP or HP gels on the market, with CP gels ranging from 10% to 22% and HP gels ranging from 3% to 10% for at-home dental bleaching.[22-25] Some studies have already compared different concentrations of CP gels for at-home bleaching, and no significant differences in terms of color change were detected when both products were used for at least two weeks.[26-28]

However, to our knowledge, there is a lack of randomized, controlled clinical trials investigating the effect of varying concentrations of HP bleaching agents on the efficacy or the risk and intensity of tooth sensitivity of at-home bleaching. Therefore, the aim of this triple-blind, controlled and parallel, randomized clinical trial was to evaluate the color change and risk and intensity of tooth sensitivity of at-home bleaching performed with HP gel in concentrations of 4% and 10%.

METHODS AND MATERIALS

Ethics Approval and Protocol Registration

This clinical investigation was approved by the ethics committee from the local university (Protocol Number 1.009.881), and it was registered in the Brazilian clinical trials registry under the identification number RBR-45xmzj. We prepared this article using the protocol established by the Consolidated Standards of Reporting Trials statement.[29]

Trial Design, Settings, and Locations of Data Collection

This was a triple-blind, controlled, parallel, randomized clinical trial, in which the patient, operator and evaluator were blinded to the group assignment. This study was performed between March 2015 and September 2015 in the clinics of the school of dentistry at the local university.

Recruitment

Two weeks before the bleaching procedures, all the volunteers, who were patients and students seeking treatment at the clinic of the dental school, received dental prophylaxis with pumice and water in a rubber cup and signed an informed consent form. Recruitment was performed by placing a written advertisement on the university walls.

Eligibility Criteria

Patients included in this clinical trial were at least 18 years old and had good general and oral health. The participants had six caries-free maxillary anterior teeth, without restorations and with no periodontal disease. The superior central incisors

were shade A2 or darker as judged by comparison with a value-oriented shade guide (Vita Classical, Vita Zahnfabrik, Bad Säckingen, Germany). Two calibrated investigators performed this evaluation. They were required to have an agreement of at least 85% (κ statistic) before beginning the study evaluation.

Participants with anterior restorations or a dental prosthesis, orthodontics apparatus, or severe internal tooth discoloration (tetracycline stains, fluorosis, and pulpless teeth) were not included in the study. In addition, pregnant/lactating women, participants with any other pathology that could cause sensitivity (such as recession, dentin exposure, or the presence of visible cracks in teeth), smokers, bruxers, or participants who had previously undergone tooth-whitening procedures were also excluded.

Sample Size Calculation

The primary outcome of this study was the absolute risk of tooth sensitivity (TS). A preliminary study with 20 patients using the 10% hydrogen peroxide gel (White Class Calcium, FGM, Joinville, Brazil) showed an absolute risk of TS of 70%. Thus, a minimal sample size of 38 participants per group were required to have a 90% chance of detecting, as significant at the two-sided 5% level, a decrease in the primary outcome measure from 70% to 35% using a low-concentration HP gel.

A secondary sample size calculation for the secondary outcome color change was also performed. For color variation (ΔE) a minimum of 33 participants per group would be required to exclude a mean difference of 3.0 in the ΔE, with 90% power and 5% α, considering that the standard deviation of ΔE is approximately 3.5 units. This limit of equivalence (difference of means) was because only a ΔE greater than 3.0 is considered clinically perceptible.

Randomization and Allocation Concealment

We used blocked randomization (block sizes of two and four) with an equal allocation ratio to form the allocation list for the two comparison groups. The randomization list was prepared in a software program freely available online (http://www.sealedenvelope.com). Opaque, sealed, and consecutively numbered envelopes containing the identification of the groups were prepared by a third person who was not involved in the research protocol. An envelope was only opened immediately before the beginning of the bleaching procedure.

Study Intervention

Alginate impressions (Avagel, Dentsply, Petrópolis, Brazil) were made of each participant's maxillary arch, and after disinfection these were filled with dental stone (Asfer, Asfer Indústria Química Ltda, São Caetano do Sul, Brazil). A 0.9-mm soft vinyl material (Whiteness Placas para Moldeiras, FGM) was used to fabricate the custom-fitted tray that would hold the whitening gel in the Plastivac P7 (BioArt, São Carlos, Brazil). The excess material from the labial and lingual surfaces was trimmed to 1 mm from the gingival junction.

At this time, group assignments were revealed and each patient received the bleaching tray and his or her respective bleaching product. We instructed all participants to wear the tray with the bleaching agent for 30 minutes twice a day for 14 days. We instructed the participants to remove the tray after each bleaching period, wash it with water, and brush their teeth as usual.

As a measure of adherence to the experimental protocol, participants were given a diary in which they were asked to take note of the number of times they used the tray during the treatment. If they had worn the bleaching tray 28 times, this would result in a 100% adherence to the protocol. We also provided verbal instructions about oral hygiene, encouraging participants to brush their teeth regularly with fluoridated toothpastes without whitening components.

Color Evaluation

Two calibrated evaluators with agreement of at least 85% determined by weighted κ statistics recorded the shade of each participant's teeth at baseline, during treatment (after the first and second week of bleaching treatment), and one month postbleaching. In the event of disagreements between the examiners during shade evaluation, a consensus was reached through discussion.

The color evaluation was performed with the use of two value-oriented shade guide units: Vita Classical (Vita Zahnfabrik)[30,31] and Vita Bleached-guide 3D-MASTER (Vita Zahnfabrik)[6,32,33] and with the aid of a spectrophotometer (Easyshade, Vita Zahnfabrik).[25,31]

For color evaluation with the Vita Classical scale, the 16 tabs of the shade guide were arranged from the highest (B1) to the lowest (C4) value. Although this scale is not linear in the truest sense, for the purpose of analysis, the changes were treated as though they represented a continuous and approx-

imately linear ranking. The Vita Bleachedguide 3D-MASTER contains lighter shade tabs and is already organized from the highest (0M1) to the lowest (5M3) value. The measurement area of interest for shade matching was the middle one-third of the facial surface of the anterior central incisor.

The two examiners, blinded to the allocation assignment, scheduled these patients for bleaching and evaluated their teeth against the shade guide at the different time assessments. Color changes were calculated from the beginning of the active phase through 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.

For the color evaluation with spectrophotometer Vita Easyshade (Vita Zahnfabrik), an impression of the maxillary arch was taken with dense silicone paste (Speedex Putty, Coltene, Rio de Janeiro, Brazil). The impression was extended to the maxillary canine and served as a standard color measurement guide for the spectrophotometer. For each dental component to be evaluated, a window was created on the labial surface of the molded silicone guide using a metal device with a radius of 6 mm and well-formed borders. The shade was determined using the parameters of the Easyshade device where it indicated the following values: L*, a*, and b*, in which L* represented the value from 0 (black) to 100 (white) and a* and b* represented the shade, where a* was the measurement along the red-green axis and b* was the measurement along the yellow-blue axis. The color comparison before and after treatment was given by differences between the two colors (ΔE), which was calculated using the formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Commission Internationale de l'Eclairage).

TS Evaluation

Participants were asked to keep a daily record of whether they experienced sensitivity. The patient was asked to indicate the numerical value of the degree of sensitivity using a 5-point numeric rating scale (NRS) where 0 = *none*, 1 = *mild*, 2 = *moderate*, 3 = *considerable*, and 4 = *severe* [6,28,34] and also to express their pain intensity using a visual analog scale (VAS).[6,35,36] This scale was a 10-cm horizontal line with scores of 0 and 10 at the ends, where 0 = *no sensitivity* and 10 = *severe sensitivity*. The patient marked, with a vertical line across the horizontal line of the scale, the intensity of the TS. Then, the distance in millimeters from the zero end was measured with the aid of a millimeter ruler.

The worst score from the NRS and the highest numeric value obtained in the VAS during all bleaching treatments were considered for statistical purposes, so that only a single value was taken from the two-week treatment. The values were arranged into two categories: absolute risk of TS, which represented the percentage of patients who reported TS at least once during treatment, and the overall TS intensity.

Blinding

This was a triple-blind study in which the operator, patient, and evaluator were not aware of the group assignment. To maintain blinding, the two bleaching products (4% [HP 4] or 10% [HP 10] HP [White Class, FGM]) were transferred to identical whitening syringes of the same color and were identified as codes "A" or "B." Only the research coordinator knew the coding system.

Statistical Analysis

The analysis followed the intention-to-treat protocol and involved all participants who were randomly assigned (Figure 1).[29] The statistician was blinded to study groups. The absolute risks of bleaching-induced TS were compared by χ^2 test. The intensity of TS from both pain scales were compared using the Mann-Whitney test.

At each time assessment, the color change data in ΔSGU from the two shade guide units were compared with the Mann Whitney test. The ΔE data (groups vs assessment time) were submitted to a two-way repeated measures analysis of variance with the assessment time being the repeated factor. After this, the post hoc Tukey test was used for pairwise comparisons. In all statistical tests, the significance level was 5%.

RESULTS

Characteristics of Included Participants

A total of 251 participants were examined according to the inclusion and exclusion criteria (Figure 1), but only 78 participants remained for the clinical trial. The baseline color of the participants' teeth was similar in shade guide units as was their mean age in years (Table 1). The distribution of the genders in both groups was quite similar (Table 1). No hypothesis testing was performed for baseline features because any difference between these features is attributed to chance alone.

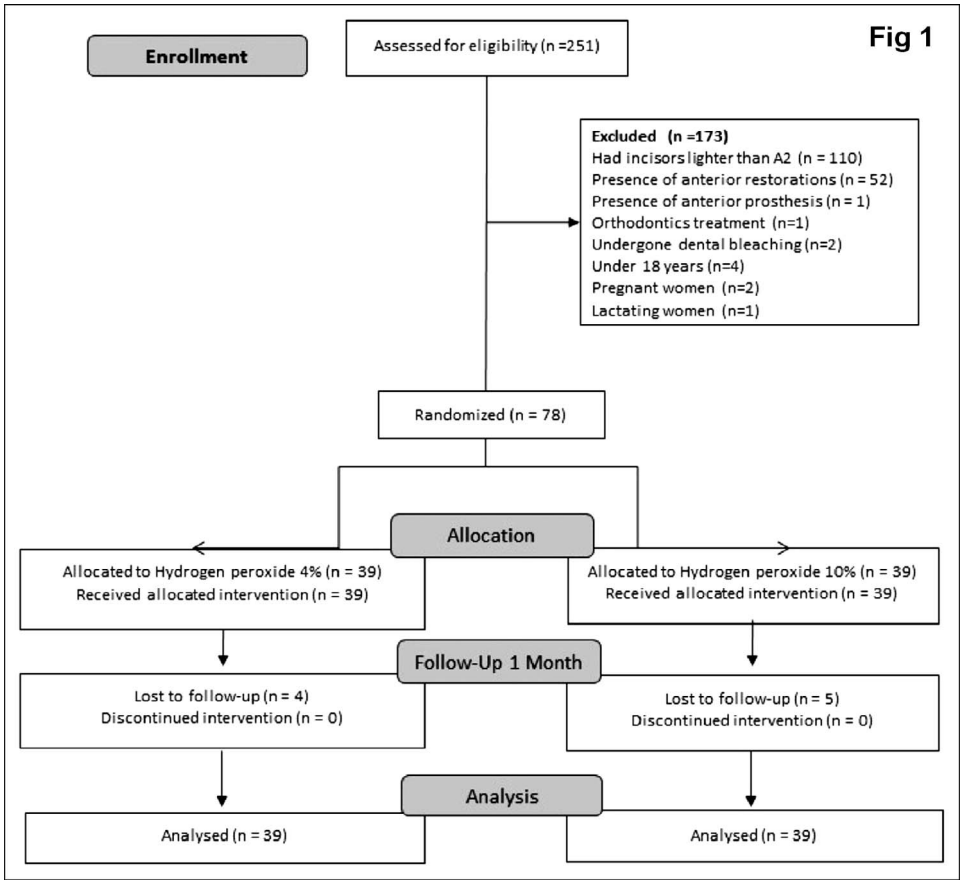

Figure 1. *Flow diagram of study design phases including enrollment and allocation criteria.*

Adherence to the Protocol

The adherence to protocol was 88% in the HP 10 and 85% in the HP 4, meaning that patients did not use the bleaching tray at times during the two-week protocol. All participants attended the recall visits during the bleaching protocol. Nine participants did not attend the one-month recall visit. For these participants, the last observation was carried forward for statistical purposes to keep the intention-to-treat analysis.[29] Figure 1 depicts the participant flow in the different phases of the study design.

Tooth Sensitivity

In regard to the absolute risk of bleaching-induced TS, a significant difference was observed between groups as seen in Table 2 (χ^2 test, p=0.04). In HP 10, there were 25 patients who reported tooth sensitivity of some degree during bleaching, and in HP 4, only 15 patients reported some degree of pain during treatment. The risk ratio, along with the 95% confidence interval, is also evidence that the use of the HP 10 produced significantly higher risk of bleaching-induced TS than did HP 4.

According to the NRS pain scale, two patients from HP 10 reported severe TS, whereas none from HP 4 reported this degree of pain. The highest level of TS

Table 1: *Demographic Characteristics of the Participants*

	HP 10	HP 4
Baseline color, SGU (mean ± SD)	5.6 ± 0.9	6.2 ± 2.0
Age, y (mean ± SD)	23.6 ± 5.9	24.3 ± 9.1
Gender (female; %)	64.1	53.8

Abbreviations: HP 4, group with 4% hydrogen peroxide; HP 10, group with 10% hydrogen; SGU, shade guide unit measured by Vita Classical.

Table 2: *Comparison of the Number of Patients Who Experienced Tooth Sensitivity During the Bleaching Regimen in Both Groups Along With Absolute Risk and the Risk Ratio**

Treatment	Tooth Sensitivity (Number of Patients)		Absolute Risk (95% CI)	Risk Ratio (95% CI)
	Yes	No		
HP 10	25	14	64 (48–77)	0.6 (0.38–0.95)
HP 4	15	24	38 (25–54)	

Abbreviations: HP 4, group with 4% hydrogen peroxide; HP 10, group with 10% hydrogen.
** χ^2 test (p=0.04).*

Table 3: *Means and Standard Deviations and Medians (Interquartile Range) of the Tooth Sensitivity Intensity Using Both Sensitivity Scales as Well as Statistical Analysis*

Pain Scales	Means and Standard Deviations		Medians and Interquartile Range		*p*-value*
	HP 10	HP 4	HP 10	HP 4	
Numeric rating scale (0-4)	0.8 ± 0.9	0.5 ± 0.8	1 (0 – 1)	0 (0 – 1)	0.02
Visual analog scale (0-10)	1.2 ± 2.1	0.7 ± 1.5	0.5 (0 – 1.3)	0 (0 – 0.65)	0.03

Abbreviations: HP 4, group with 4% hydrogen peroxide; HP 10, group with 10% hydrogen.
** Mann-Whitney test.*

reported in the HP 4 group was considerable and was reported by two patients. Regarding the TS intensity (Table 3), significant differences between the two groups were observed with the two pain scales used in this study ($p=0.02$ and $p=0.03$, for NRS and VAS, respectively), showing greater intensity of TS for HP 10.

Color Evaluation

Significant whitening was observed in both study groups under the subjective and objective evaluation methods. Most of the whitening occurred within the first week of bleaching, as can be observed by the three different instruments used for evaluation of color changes (Table 4). The subjective results (Vita Classical: $p=0.38$; Vita Bleachedguide: $p=0.11$) and the objective evaluation with the spectrophotometer ($p=0.27$) indicates that there was no significant difference between groups after bleaching (Table 4).

Although the three tools used for color evaluation did not produce identical results, they tended to show a higher whitening degree for the HP 10 in the first week of bleaching (Vita Classical and Vita Bleachedguide) and/or the second week of bleaching (Vita Bleachedguide and spectrophotometer) than the HP 4. However, at one-month postbleaching, no significant difference was observed between both products for any of the instruments used for color evaluation, meaning that at the end of the bleaching protocol, similar color changes could be achieved with the two HP concentrations ($p>0.05$).

DISCUSSION

We have used three different tools for evaluation of color changes in the present study to obtain a more reliable evaluation of the whitening changes that occurred during bleaching. The Vita Classical shade guide is widely used in clinical studies of dental bleaching and therefore allows comparison of the results with earlier clinical trials in this field. It was already demonstrated to be a valid method, with good reliability for differentiating between dark and light colors.[37] However, this shade guide was not specifically designed for tooth whitening assessment: The tabs in the shade guide are nonlinear and it lacks color uniformity, and some overlap between similar colors provides little resemblance to reality.[25,38]

That is why we have also used the more recent Vita Bleachedguide 3D-MASTER scale, developed

Table 4: *Means and Standard Deviations of ΔSGU Obtained With the Vita Classical and Vita Bleachedguide 3D-MASTER and ΔSGU Obtained With the Spectrophotometer Vita Easyshade at Different Periods as Well as the Statistical Analysis*

	Periods	HP 10	HP 4	Mean difference (95% CI)	*p*-value*
ΔSGU (Vita Classical)	1 wk	3.7 ± 1.2	3.0 ± 1.3	0.7 (0.13 to 1.27)	0.01
	2 wk	4.2 ± 0.9	4.0 ± 1.3	0.2 (−0.31 to 0.71)	0.24
	1 mo postbleaching	4.1 ± 0.9	4.1 ± 1.3	0.0 (−0.51 to 0.51)	0.38
SGU (Vita Bleachedguide 3D-MASTER)	1 wk	5.1 ± 2.3	3.7 ± 1.6	1.4 (0.49 to 2.31)	0.006
	2 wk	6.6 ± 2.6	5.3 ± 1.8	1.3 (0.28 to 2.32)	0.03
	1 mo postbleaching	6.1 ± 2.5	5.0 ± 2.3	1.1 (0.00 to 2.20)	0.11
ΔE	1 wk	7.4 ± 3.6	6.5 ± 3.2	0.9 (−0.06 to 2.46)	0.23
	2 wk	9.0 ± 4.2	6.8 ± 3.0	2.2 (0.53 to 3.87)	0.01
	1 mo postbleaching	8.4 ± 3.5	7.9 ± 4.5	0.5 (−1.34 to 2.34)	0.27

Abbreviations: ΔE, change in color; ΔSGU, change in shade guide units; HP 4, group with 4% hydrogen peroxide; HP 10, group with 10% hydrogen; SGU, shade guide units.
** Mann-Whitney test.*

for bleaching research purposes. This newer scale has more tabs lighter than B1, which expands the degree of measurements of the shade guide. Although Vita Bleachedguide 3D-MASTER was developed in 2007, only a few studies have used this new shade guide for bleaching research.[6,33,39-42] In the present study, the use of Vita Bleachedguide 3D-MASTER seemed to be more sensitive because it was the only color measurement tool able to detect the subtle but significant differences between the groups after one and two weeks of at-home bleaching and one month postbleaching. The spectrophotometer provides an objective, consistent and reliable monitoring of color change that is less affected by observer training and variability.[38,43]

These three instruments used for color evaluation were not unanimous in their findings. They showed variations in their results. However, an overall trend could be observed. When differences were detected between groups during the first and second week of bleaching, HP 10 showed a higher degree of whitening, meaning that a higher HP concentration may initially boost the whitening outcome. However, this initial advantage is lost during the two-week treatment because all color measurement instruments demonstrated the same degree of color change for the two bleaching products after the 2-week protocol, with a bleaching of approximately four and six units of color on the Vita Classical and Vita Bleachedguide scale, respectively, and a ΔE color variation of approximately eight units when a spectrophotometer was used.

This corroborates with the results of several clinical trials[12,28,44] that compared different concentrations of CP. For example, in the study of Meireles and others,[28] no difference in effectiveness between 10% and 16% CP were observed after a three-week protocol, but teeth exposed to 16% CP became whiter first after the first week of treatment. The same was observed by Braun and others[44] and Krause and others.[13] In both studies, the authors observed faster whitening when 17% CP was used, but at the end of the bleaching treatment in a one-week protocol, the 17% CP showed the same degree of whitening produced by the 10% CP.[13,44]

In both groups, though at different rates, bleaching-induced TS was observed. HP can pass easily through the enamel and dentin, and this phenomenon results in pulp inflammation, with release of inflammatory mediators and pulp sensory nerve stimuli.[45] The kinetics of degradation of delivered HP is a very fast process, mainly because HP-based products are very unstable and release the majority of their active HP in 60-75 minutes.[20,21]

However, one could observe that the application of 10% HP produced a higher risk of TS than did 4% HP. The absolute risk of TS of the HP 10 group was 64%, which is approximately double the one detected with the HP 4 group. As expected, the higher the initial concentration of bleaching agent, the higher the amount of HP that reaches the pulp chamber and the aggression to the pulp cells.[46,47] That is why in-office bleaching gels have a higher overall TS intensity than at-home bleaching products[6] and why highly concentrated CP bleaching agents (20%-22%) yielded higher levels of TS when compared with the traditional 10% CP.[7,11,12]

Unfortunately, most of the information we obtain from the literature about HP bleaching agents for at-home bleaching is from bleaching strips and not products for tray delivery, which prevents us from further comparisons.[32,48-51] In a recent systematic review that compared CP and HP bleaching products for at-home bleaching,[52] nine of the 13 selected papers used strip-based HP products. Although the HP concentration in strips and in a tray delivery system can be the same, the amount of HP that contacts the enamel is smaller when delivered in strips than in trays, and therefore this may yield different risks of TS.[53,54] However, further clinical trials comparing HP-based products with same concentrations in strips and in a tray delivery system should be conducted.

Despite the promising results presented in this study, more clinical studies are needed to evaluate the clinical effectiveness and adverse effects using protocols with shorter application times and lower HP concentrations for at-home bleaching.

CONCLUSIONS

It can be concluded that both HP concentrations (4% and 10%) were equally effective after two weeks of at-home bleaching. The gel with 4% HP showed lower risk and intensity of TS.

Acknowledgements

This study was partially supported by the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) under grants 304104/2013-9 and 305588/2014-1.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the State University of Ponta Grossa, Brazil. The approval code for this study is 1.009.881.

Conflict of Interest

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

(Accepted 20 May 2017)

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Eighteen-month Clinical Study of Universal Adhesives in Noncarious Cervical Lesions

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Clinical Relevance

Some of the currently available universal adhesives may present similar clinical performance when applied in the self-etch and etch-and-rinse modes. Yet clinicians should be aware that use in the etch-and-rinse application mode tends to result in fewer stained margins when compared to the self-etch application mode.

SUMMARY

Objective: To evaluate the clinical performance of Scotchbond Universal (3M Oral Care) and Prime & Bond Elect (Dentsply Sirona) in the restoration of noncarious cervical lesions (NCCLs).

Methods and Materials: This was a randomized controlled clinical trial involving 63 subjects. Two hundred and three NCCLs were restored using Scotchbond Universal and Prime & Bond Elect using both an etch-and-rinse and a self-etch technique. Lesions were notch-shaped NCCLs, and the restorations were placed without any mechanical retention. Restorations were finished immediately after placement and scored with regard to retention, marginal discoloration, marginal adaptation, and secondary caries. Similar assessment of the restorations was performed 18 months after placement. Logistic regression was performed for each outcome separately with a compound symmetric variance-covariance structure assumed to consider a correlation of restorations within subjects. All analyses were conducted using SAS version 9.4 (SAS Inc).

Results: One hundred and fifty-eight teeth (77.8% of the restorations placed) in 46 subjects (73% of subjects enrolled) were available for the 18-month follow-up. A statistically significant difference was reached only for the comparison Scotchbond Universal/self-etch (SU_SE) and Prime & Bond Elect/etch-and-rinse (PBE_E&R) groups (p=0.01), where

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DOI: 10.2341/16-320-C

a restoration with SU_SE was 66% less likely to maintain a score of Alpha for marginal discoloration than a restoration performed with PBE_E&R.

Conclusions: Scotchbond Universal and Prime & Bond Elect presented acceptable clinical performance after 18 months of clinical service. However, Scotchbond Universal, when applied with a self-etch approach, did demonstrate a relatively high level of marginal discoloration when compared to the other groups.

INTRODUCTION

Adhesion of varying degrees to both dentin and enamel can be accomplished by using etch-and-rinse and self-etch techniques.[1,2] Etch-and-rinse adhesive systems rely on the initial application of phosphoric acid to demineralize dentin and enamel. After rinsing, the demineralized surfaces that remain allow for subsequent infiltration of a low-viscosity resin to achieve micromechanical retention.[1] This approach is common to both two-step etch-and-rinse adhesives (primer and adhesive in one bottle) and three-step etch-and-rinse adhesives (primer and adhesive applied separately).

Clinical challenges that may compromise the longevity of restorations performed with etch-and-rinse adhesive systems include 1) the potential for errors during application because of the multiple steps (particularly for three-step materials) and 2) maintenance of an adequately hydrated collagen network after the dentin is demineralized by phosphoric acid.[3-5] To allow for optimal resin infiltration into the demineralized collagenous network, dentin must not be overdried and desiccated.[5]

Meanwhile, self-etch adhesive systems, which do not include phosphoric acid pretreatment of dentin and enamel, can be found in one- or two-step application modes.[2] Two-step self-etch adhesive systems have been available for many years and have been shown to be clinically reliable.[6,7] On the other hand, one-step self-etch adhesive systems, which normally combine all adhesion steps in one single bottle, have demonstrated questionable clinical success.[7-9] In order to provide adequate conditioning of the tooth structures, one-step adhesive systems require significant amounts of water to ionize their acidic monomers. An inverse correlation between hydrophilicity of materials and stability of adhesive interfaces has been continuously demonstrated in *in vitro* studies, thereby raising concerns for longevity of the bonds.[6,8-11]

Recently, adhesive systems that can be applied using either the etch-and-rinse or the self-etch technique, namely, universal adhesives, have been developed.[12] These materials can be used with or without pretreatment of dentin and enamel with phosphoric acid, even though selective etching of enamel is often recommended, and the adhesive system can be applied to dry or wet dentin.[13] Universal adhesives contain functional monomers in their compositions that may enhance bonding through chemical adhesion to tooth structures. One of these monomers, namely, 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), has been part of the composition of dental adhesives for decades.[14] Its affinity for calcium in hydroxyapatite, which is amplified and stabilized by the low solubility of the calcium salt of the acidic molecule, makes it a desirable component for adhesive systems.[14-17] However, the ability of functional monomers to properly condition the enamel structure in order to have restorations free of marginal staining has been questioned.[13,18] The pH of universal adhesives is directly related to the ability of the material to sufficiently demineralize tooth structure and is in the mild range.[19,20] This feature makes it ideal for adhesion to dentin but sometimes inadequate for optimal enamel bonding.[19-22] Clinical data demonstrating that universal adhesives can be applied without phosphoric acid pretreatment of enamel are needed despite favorable *in vitro* data suggesting that they do adhere well to both dentin and enamel substrates.[12,23-26]

Clinical evaluation of adhesive systems is often performed using noncarious cervical lesions (NCCLs).[7] The inherent characteristic of NCCLs. including increased sclerosis of the dentin substrate, occlusal forces that stress the cervical third of teeth, minimal retention form, and margins that not only are in enamel but also extend to dentin, make them the ideal substrate to challenge the retention ability of adhesive systems.[27] A recent clinical trial where restorations bonded with a universal adhesive system (Scotchbond Universal, 3M Oral Care, St Paul, MN, USA) in the etch-and-rinse, self-etch, or selective etching mode showed no difference in retention after 18 months of service.[18] Similarly, no difference in clinical behavior was found when the same universal adhesive was bonded to moist or dry dentin using the etch-and-rinse technique or using the self-etch or selective etching techniques after 36 months.[13] Contradicting results were found when Scotchbond Universal was applied in etch-and-rinse and self-etch modes and compared to Scotchbond

Multi-Purpose (3M ESPE).[28] In this study, Scotchbond Universal showed superior performance with regard to marginal discoloration when applied in the etch-and-rinse mode after 24 months of function.[28] Differences in clinical performance between etch-and-rinse and self-etch techniques also have been reported for Xeno Select (Dentsply Sirona, York, PA, USA). In a recent published study, Xeno Select did not fulfill the American Dental Association (ADA) criteria for full approval when used in the self-etch or the selective etching mode after six months of clinical service.[29]

The limited clinical data available and the increased popularity of universal adhesives have led us to the design of this clinical trial. The clinical performance of two universal adhesives, namely 10-MDP-containing Scotchbond Universal and Prime & Bond Elect (Dentsply Sirona) placed in NCCLs was evaluated over a period of 18 months. The null hypothesis tested was that there is no difference in clinical performance between etch-and-rinse and self-etch techniques for Scotchbond Universal and Prime & Bond Elect with regard to retention, marginal discoloration, marginal adaptation, and secondary caries.

METHODS AND MATERIALS

This study was a randomized controlled clinical trial that evaluated the clinical performance of two universal adhesives used with a bis-GMA–free nanohybrid composite resin (Kalore, GC Corporation, Tokyo, Japan) in NCCLs. The Institutional Review Board of the Federal University of Santa Catarina, Brazil, approved the study, protocol 745.430/14. Subjects were recruited via mass e-mail to faculty, staff, and students at the Federal University of Santa Catarina.

A single operator (VCR) screened all candidates in the operative dentistry postgraduate clinic at the Federal University of Santa Catarina. Adults who were in need of restoration of at least one notch-shaped NCCL and who fulfilled the inclusion/exclusion criteria were included in the study. Excluded were candidates with fewer than 20 teeth and with poor oral hygiene, uncontrolled periodontal disease, xerostomia, or known allergy to resin-based materials or who were medically compromised, pregnant, or breast-feeding. Teeth that had saucer-shaped lesions or that were nonvital, not in occlusion, or previously restored also were excluded.

The selected teeth were allocated into four groups to be restored according to the following protocols: 1) Scotchbond Universal, etch-and-rinse mode (SU_E&R); 2) Scotchbond Universal, self-etch mode (SU_SE); 3) Prime & Bond Elect, etch-and-rinse mode (PB_E&R); and 4) Prime & Bond Elect, self-etch mode (PB_SE). A member of the study team not involved with the insertions performed the randomization. The very first restoration was restored according to protocol 1. Subsequently, the randomization followed the Universal Numbering System (1-32) with the next lowest numbered tooth being restored following protocol 2 and so on. Always following in ascending order, the first tooth for the next subject was restored following the next protocol in queue. Prior to restoration, the amount (percentage) of enamel margins; restoration volume determined by height, width, and depth of the NCCL; presence of stress occlusion; gingival condition around the tooth to be restored; preoperative sensitivity; and degree of dentin sclerosis as measured by the UNC Sclerosis Scale were recorded.[30]

A total of 63 subjects with 203 NCCLs were enrolled. Subjects were 34 females and 29 males ranging from 21 to 67 years of age (42.6±12.7). A trained operative dentistry operator (VCR) performed all restorative procedures. After shade selection, moisture control was performed with retraction cord, cotton rolls, lip retractor, and low-speed suction. The internal aspect of the lesion was roughened with a round diamond (#1014, KG Sorensen, Cotia, Brazil), and no bevel or mechanical retention features were placed. Anesthesia was used as needed. The adhesive systems were applied according to the manufacturer's directions as detailed in Table 1 and the preparations restored with three increments of up to 2 mm in thickness of Kalore (GC Corporation). See Figure 1 for insertion technique. Finishing and polishing procedures were immediately performed using carbide finishing burs, Jiffy Composite Adjusters and Polishers (Ultradent Products, Inc, South Jordan, UT, USA), and Astrobrush (Ivoclar Vivadent AG, Schaan, Liechtenstein). Light-curing procedures were performed using Translux Blue (Heraeus Kulzer, Hanau, Germany) at a consistent output of 800 mW/cm^2.

Two independent and calibrated evaluators (SS and SCS) assessed the restorations immediately after placement and after 18 months of clinical service using the criteria in Table 2. Evaluations were performed using an intraoral mirror and a number 23 explorer and under operatory light. Disagreements were discussed, and a consensus rating was developed.

Table 1: *Materials, Compositions, and Application Techniques for the Universal Adhesive Systems Used*

Adhesive	Composition	Application Technique	
		Self-Etch	Etch-and-Rinse
Scotchbond Universal (3M Oral Care, St Paul, MN, USA)	10-MDP phosphate monomer, dimethacrylate resins; HEMA; methacrylate-modified polyalkenoic acid copolymer; filler; ethanol; water; initiators; silane	1. Apply adhesive with a microbrush with rubbing motion for 20 s 2. Evaporate solvents with gentle stream of air until adhesive movement no longer can be noticed 3. Light cure for 10 s	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Air-dry for 5 s 4. Apply adhesive as detailed in self-etch technique
Prime & Bond Elect (Dentsply Sirona, York, PA, USA)	Mono-, di-, and trimethacrylate resins; PENTA diketone; organic phosphine oxide; stabilizers; cetylamine hydrofluoride; acetone; water	1. Apply adhesive agitating for 20 s 2. Gently dry for 5 s 3. Light cure for 10 s	1. Apply etchant for 15 s 2. Rinse for 15 s 3. Air-dry for 5 s 4. Apply adhesive as detailed in the self-etch technique

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; PENTA, dipentaerythritol penta-acrylate monophosphate.

All outcomes were evaluated as Alpha at baseline; thus, the focus of the statistical analyses was on the change of outcome overtime, that is, A vs B and C outcomes. The primary goal of the statistical analyses was to identify the effect of adhesive protocol on the outcome of the restorations. The effects of sclerosis degree and volume of the restoration on the outcome of the restorations were also considered. Descriptive statistics were performed. Logistic regression was performed for each outcome separately, where a compound symmetric variance-covariance structure was assumed to consider a correlation of restorations within subjects. All analyses were conducted at a significance level of 5% ($p \leq 0.05$), using SAS version 9.4 (SAS Inc, Cary, NC, USA).

RESULTS

One hundred and fifty-eight teeth (77.8% of the restorations placed) in 46 subjects (73% of subjects enrolled) were available for the 18-month follow-up. Table 3 shows the descriptive statistics and Table 4 shows the logistic regression results. The outcomes retention and secondary caries were not considered due to a small number of Bravo or Charlie scores at 18 months. Likewise, protocol was not included in the model for marginal adaptation. Significant statistical difference was reached only for the comparison SU_SE and PBE_E&R ($p=0.01$), where a restoration with SU_SE is 66% less likely to maintain a score of Alpha for marginal discoloration than a restoration performed with PBE_E&R. Four of 38 and 10 of 37 restorations placed with

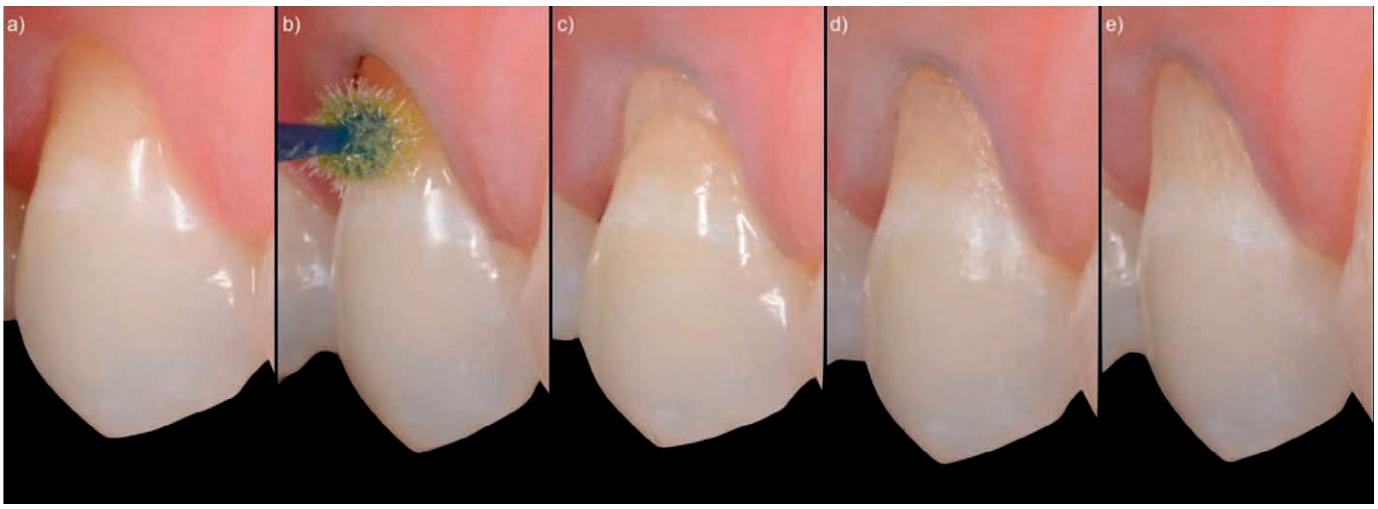

Figure 1. *Stratification of the composite resin restoration. (a): Preoperative view of the class V cavity. (b): Application of adhesive system in the self-etch mode. (c): Insertion of the first dentin increment. (d): Insertion of the second dentin increment. (e): Insertion of the enamel increment. Note that no enamel shade of composite resin was used.*

Table 2: *Modified US Public Health Service Direct Evaluation Criteria*

Criteria	Classification
Retention	Alpha = retained
	Charlie = mobile or missing
Marginal discoloration	Alfa = No discoloration at margins
	Bravo = Shallow discoloration (localized or generalized)
	Charlie = Deep discoloration (localized or generalized)
Marginal adaptation	Alpha = no visible evidence of a crevice along the margin into which the explorer will penetrate
	Bravo = visible evidence of a crevice along the margin, dentin not exposed, clinically acceptable
	Charlie = explorer penetrates into crevice, dentin exposed, clinically unacceptable
Secondary caries	Alpha = no
	Charlie = yes

PBE_E&R and SU_SE, respectively, received a score of Bravo at 18 months. Other comparisons that did not reach significant statistical differences for marginal discoloration were that 1) PB_SE is 10% less likely to maintain a score of Alpha than PB_E&R, 2) SU_SE is 42% less likely to maintain a score of Alpha than SU_E&R, 3) SU_E&R is 41% less likely to maintain a score of Alpha than PB_E&R, and 4) SU_SE is 62% less likely to maintain a score of Alpha than PBE_SE. In addition, restorations were more likely to present marginal discoloration in teeth with greater levels of dentin sclerosis, but that comparison also was not statistically significant.

DISCUSSION

The null hypothesis that there is no difference in the clinical performance between etch-and-rinse and self-etch techniques for Scotchbond Universal and Prime & Bond Elect with regard to retention, marginal discoloration, marginal adaptation, and secondary caries was accepted. Scotchbond Universal and Prime & Bond Elect had similar clinical performance, the only statistically significant difference being found for the comparison between SU_SE and PB_E&R, in which the former had a 66% less chance to maintain ideal margins with regard to marginal discoloration over time than the latter.

Overwhelming *in vitro* literature suggesting that degradation of resin-dentin bonding may affect the longevity of composite resin restorations exists.[6,31] It is believed that the collagen matrix at the adhesive interface is only partially infiltrated by the bonding resin after phosphoric acid treatment of dentin.[1,6,12,13,32] The exposed collagen would then be susceptible to degradation by matrix metalloproteinases,[33,34] ultimately resulting in failure of the adhesive interface by fatigue.[1,6,31] The clinical implications of the resin-dentin bonding degradation are somewhat unclear, as cavity preparations often have enamel margins, which do not undergo the same degradation process. Recently, the degradation of the resin-dentin bonding was disregarded as a clinical problem when the overall caries risk of the patient is under control.[35] Studies evaluating the effects of aging on the dentin bond strength of universal adhesives applied in the etch-and-rinse mode have shown no deterioration of the adhesive interface.[26,36]

On the other hand, the major issue related to self-etch adhesives seems to be enamel marginal discoloration due to the mild acidity of these adhesives when compared to phosphoric acid.[2] Several *in vitro* studies have demonstrated that the enamel bonding created by self-etch adhesives is inferior to that created by etch-and-rinse materials.[37-39] The two universal adhesives tested in this study have similar pH values (PBE=2.5 and SU=2.7).[19-21] Ultramild self-etch adhesives (pH>2.5) have limited interaction with dentin, resin tags are hardly formed, smear plugs get slightly demineralized and resin-infiltrated, and there is less potential for enamel etching with lower chemical reactivity with hydroxyapatite in enamel.[2] A meta-analysis of *in vitro* studies demonstrated that phosphoric acid etching of enamel increases the bond strength of universal adhesives to that substrate.[21]

Despite the *in vitro* evidence that enamel etching may be critical for maintenance of restorations without marginal discoloration, a recent 13-year randomized clinical trial comparing a self-etch adhesive applied to NCCLs with and without the use of selective etching of enamel showed only a minor positive effect of selective etching of enamel on marginal integrity and absence of marginal discoloration. The differences were not statistically significant.[40] Our study showed no significant difference in clinical performance between universal adhesives applied using the etch-and-rinse and self-etch technique in the short term. Yet 10 of 37 restorations placed with SU_SE showed marginal discoloration after 18 months, which may be considered a high incidence in such a short period of time. Despite the fact that 16.6% (26 of 157) and 5.7% (9 of 157) of the restorations presented a Bravo score for marginal discoloration and marginal adaptation, respectively, at 18 months, the use of phosphoric acid on the

Table 3: *Descriptive Statistics for a Total Number of 158 Restorations Placed in 46 Subjects. Data Are Listed as Percentage After 18 Months for the Outcomes of Retention, Marginal Discoloration, Marginal Adaptation, and Secondary Caries and Frequency for the Covariates of Tooth Type, Dentin Sclerosis, and Mean Volume (±SD) and for Restorations Evaluated for Each Adhesive. Missing Restorations (Unavailable for Evaluation) Were Excluded From the Calculations of the Percentage Shown*

Type	Variable	Total (n=203)	SU_E&R (n=52)	SU_SE (n=50)	PBE_E&R (n=50)	PBE_SE (n=51)
Outcome	Retention					
	Alpha	157 (99.4%)	42	37	38	40
	Charlie	1 (0.6%)	0	0	0	1
	Missing	45	10	13	12	10
	Marginal discoloration					
	Alpha	131 (83.4%)	35	27	34	35
	Bravo	26 (16.6%)	7	10	4	5
	Charlie	0 (0.0%)	0	0	0	0
	Missing	46	10	13	12	11
	Marginal adaptation					
	Alpha	148 (94.3%)	38	35	38	37
	Bravo	9 (5.7%)	4	2	0	3
	Charlie	0 (0.0%)	0	0	0	0
	Missing	46	10	13	12	11
	Secondary caries					
	Alpha	157 (100%)	42	37	38	40
	Charlie	0 (0.0%)	0	0	0	0
	Missing	46	10	13	12	11
Covariate	Tooth type					
	Canine	23	9	5	2	7
	Incisor	9	2	2	2	3
	Molar	19	6	5	4	4
	Premolar	107	25	25	30	27
	Dentin sclerosis					
	1	73	19	14	22	18
	2	68	21	19	13	15
	3	15	1	4	2	8
	4	2	1	0	1	0
	Volume (mm^3)	11.0 ± 8.7	10.1 ± 5.8	12.8 ± 11.0	11.5 ± 10.5	9.8 ± 6.5

Abbreviations: SU_E&R, Scotchbond Universal/etch-and-rinse; SU_SE, Scotchbond Universal/self-etch; PBE_E&R, Prime & Bond Elect/etch-and-rinse; PBE_SE, Prime & Bond Elect/self-etch.

preparation failed to alter the outcome of the restorations. PB_SE was 10% less likely to maintain a score of Alpha than PB_E&R, while SU_SE was 42% less likely to maintain a score of Alpha than SU_E&R with regard to marginal discoloration. It should be pointed out that marginal discoloration often occurs due to excess material at the margins of the restoration and can be solved by repolishing.[41]

Previous clinical studies with longer follow-up periods than our study have shown inferior marginal discoloration or adaptation over time. In 2015, Lawson and others[28] showed no statistical differences between Scotchbond Universal applied in the etch-and-rinse and self-etch techniques with regard to secondary caries and marginal adaptation. However, the etch-and-rinse application of the adhesive resulted in fewer cases of marginal discoloration at 24 months. Likewise, increased marginal staining was found for Scotchbond Universal when applied using the self-etch technique compared to the etch-and-rinse technique after 36 months of service in another controlled clinical trial.[13]

Studies evaluating the performance of dental restorative materials have used for decades some variation of the US Public Health Service (USPHS) criteria.[42-44] These criteria, which have shown to be effective in determining the clinical performance of restorative materials over time, may not be specific

Table 4: *Results of Logistic Regression for Marginal Discoloration and Marginal Adaptation Modeling the Probability of Maintaining Alpha Scores, With Compound Symmetry Correlation Structure*

Outcomes	Covariates	Effect	OR	95% CI		z	p
Marginal discoloration	Protocol	PBE_SE vs PBE_E&R	0.90	0.24	3.36	−0.16	0.87
		SU_E&R vs PBE_E&R	0.59	0.20	1.76	−0.94	0.35
		SU_SE vs PBE_E&R	0.34	0.15	0.79	−2.53	0.01
		SU_E&R vs PBE_SE	0.66	0.18	2.42	−0.62	0.53
		SU_SE vs PBE_SE	0.38	0.11	1.27	−1.57	0.12
		SU_SE vs SU_E&R	0.58	0.25	1.33	−1.29	0.20
	Sclerosis	2 vs 1; 3 vs 2; 4 vs 3	0.56	0.29	1.08	−1.73	0.08
		3 vs 1; 4 vs 2	0.31	0.08	1.17		
		4 vs 1	0.17	0.02	1.27		
	Volume		1.001	0.95	1.05	0.04	0.97
Marginal adaptation	Sclerosis	2 vs 1; 3 vs 2; 4 vs 3	0.70	0.23	2.11	−0.64	0.52
		3 vs 1; 4 vs 2	0.49	0.05	4.46		
		4 vs 1	0.34	0.01	9.40		
	Volume		1.04	1.06	0.66	−0.42	0.67

Abbreviations: OR, odds ratio; CI, confidence interval; PBE_SE, Prime & Bond Elect/self-etch; PBE_E&R, Prime & Bond Elect/etch-and-rinse; SU_E&R, Scotchbond Universal/etch-and-rinse; SU_SE, Scotchbond Universal/self-etch.

enough to identify minor differences among the very similar and extensively developed modern restorative materials. This observation led to the development of the Federation Dentaire Internationale (FDI) criteria in 2007.[45] The current study, which used the USPHS modified criteria, failed to demonstrate significant differences when comparing the application techniques, namely, etch-and-rinse and self-etch techniques, of two universal adhesives. Similarly, no differences in marginal adaptation and discoloration were found in another recently published controlled clinical trial comparing Scotchbond Universal applied following different techniques. Interestingly, a significant increase in marginal discrepancies when the material was used in the self-etch technique was found when the FDI criteria were applied in that study.[18]

Aside from containing 10-MDP, which may enhance adhesion to tooth structures through chemical adhesion to hydroxyapatite in a process called nanolayering,[14,15,18,46,47] SU contains polyalkenoic acid copolymer in its composition. The ionic bond between the carboxyl groups in the polyalkenoic acid and hydroxyapatite in enamel and dentin may have aided the adhesion of SU to the NCCLs in this study, as demonstrated in an *in vitro* study.[24] Prime & Bond Elect, on the other hand, contains dipentaerythritol penta-acrylate monophosphate (PENTA) in its composition. To the best of the authors' knowledge, there is no *in vitro* study demonstrating the effects of PENTA on adhesion. In a recently published *in vitro* study, PB presented the lowest dentin bond strengths when adhesives were applied according to the manufacturers' instructions.[48]

Some limitations of this study that are worth mentioning are 1) the relatively low sensitivity of the modified USPHS evaluation criteria used and 2) the placement of all restorations by the same operator. Further evaluation of the restorations placed in this study is planned, and an updated report should be available in the near future.

CONCLUSIONS

Scotchbond Universal and Prime & Bond Elect presented acceptable clinical performance after 18 months of clinical service. However, it should be noted that Scotchbond Universal used with a self-etch approach did exhibit a fairly high incidence of marginal discoloration for this short clinical time period.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Federal University of Santa Catarina. The approval code for this study is 745.430/14.

Conflict of Interest

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

(Accepted 7 January 2017)

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Does the Use of a "Walking Bleaching" Technique Increase Bone Resorption Markers?

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Clinical Relevance

Intracoronal bleaching (nonvital bleaching) using a "walking bleaching" technique generates increased markers related to bone resorption.

SUMMARY

Objective: This randomized clinical trial evaluated the effect of 35% hydrogen peroxide in comparison with 37% carbamide peroxide in a nonvital bleaching technique of "walking bleaching" (four sessions of treatment) on periodontal markers: nuclear factor kappa B-ligand (RANK-L—process of root resorption marker) and interleukin 1β (IL-1β—inflammatory response marker).

Methods and Materials: Fifty volunteers presenting with discoloration of nonvital teeth and endodontic treatment in good condition participated. Fifty teeth were randomly divided into two study groups according to bleaching gel: HP = 35% hydrogen peroxide (n=25) and 37% carbamide peroxide (n=25). Nonvital bleaching was performed with a walking bleaching technique consisting of four sessions of bleach application. Gingival crevicular fluid samples were taken in order to quantify the RANK-L and IL-1β levels by

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DOI: 10.2341/16-334-C

enzyme-linked immunosorbent assay. Samples were obtained from six periodontal sites for each bleached tooth: three vestibular and three palatine (mesial, middle, and distal) at seven time periods: baseline, after each of the four sessions of nonvital bleaching, at one week, and at one month after nonvital bleaching. Tooth color variations were analyzed in each session by VITA Bleachedguide 3D-MASTER (ΔSGU).

Results: Significant increments in the RANK-L and IL-1β levels were detected in each evaluated time compared with baseline ($p<0.05$); however, no differences were detected between hydrogen peroxide and carbamide peroxide on increments of the biomarkers studied. The change of color was effective for both nonvital bleaching therapies ($p<0.05$).

Conclusions: Nonvital bleaching induced a significant increment in the RANK-L and IL-1β levels in periodontal tissues around bleached, nonvital teeth.

INTRODUCTION

The intracoronal bleaching technique has traditionally been used to solve esthetic problems of nonvital tooth discoloration since it is a minimally invasive, fast, and effective treatment. In general, gels of sodium perborate, carbamide peroxide, and hydrogen peroxide are used to treat discolored teeth.[1] For all these products, the active agent is hydrogen peroxide, which whitens by means of an oxidation chemical reaction.[2] Bleaching of a discolored tooth positively impacts esthetic self-perception and psychosocial impact,[3,4] as do other vital bleaching systems,[5] and even patient satisfaction and correlation with personality styles.[6,7]

However, one of the adverse effects of intracoronal bleaching that has been reported is external root resorption, which is increasingly rare but still apparent when tooth loss, which occurs quite frequently, is achieved.[1] The incidence mentioned in the literature varies greatly, from 1% to 13%.[8-11] Unfortunately, the etiology of root resorption has yet to be comprehensively explained. It is known that the high oxidative power of hydrogen peroxide can alter the histological and morphological properties of tooth structure.[12-14] Also, hydrogen peroxide has a low molecular weight of H_2O_2 and is able to diffuse through the tooth structure and stay in periodontal tissues.[15] An extra-root effect produced by free-radical peroxide has produced periodontal cell cytotoxicity and increased pH in the surface of the root.[16]

Recent studies have shown an increase in the activity of inflammatory cytokines and metalloproteinases when using bleaching techniques.[12,17] These studies suggest that this could also have an effect on the levels of markers of bone destruction, considering that they would have spread to the periodontal tissues. The most important marker of destruction that has been extensively studied is the receptor activator of nuclear factor kappa B-ligand (RANK-L), which is also associated with regulating the process of root resorption.[18,19] Interleukin 1β (IL-1β) plays a central role in immune and inflammatory responses and the bone remodeling process. It is a cytokine that is present in most of the inflammatory processes, and its increase has been linked to a number of pathological processes, such as periodontitis and marginal bone loss.[20]

There are no reports of associations between increased biomarkers of bone resorption and nonvital whitening using the "walking bleaching" technique. Evidence remains poor, and there are no studies that explain the process by which hydrogen peroxide could act on the processes of tissue destruction surrounding the tooth.

Hydrogen peroxide generates a cytotoxic and tissue-damaging effect. There are reports on the relationship between lower concentrations and less negative effects;[21] therefore, considering that carbamide peroxide degrades to one-third of its concentration being hydrogen peroxide, there may be a lowered effect on periodontal tissue compared with a hydrogen peroxide gel of similar concentration.

Although there is no consensus about the proper amount of hydrogen peroxide to be used with the walking bleaching technique, lower concentrations are typically used since they have a lower potential to alter the histological and morphological properties of the tooth structure.[12-14] However, to our knowledge, no previous published clinical studies have evaluated this issue. Therefore, the aim of this randomized clinical trial was to evaluate the effect of 35% hydrogen peroxide and 37% carbamide peroxide in a nonvital walking bleaching technique (four sessions of treatment) on periodontal markers RANK-L and IL-1β. The first null hypothesis was that the two gels do not have any effect on the induction of IL-1β and RANK-L at different times.

METHODS AND MATERIALS

Fifty volunteers were selected with one nonvital tooth with discoloration. Prior to the start of the

study, patients signed an informed consent form. They received a prophylaxis brush and a slurry of pumice and water. In addition, oral hygiene instructions were given to standardize the oral conditions of each volunteer and to control for basal periodontal health status.

Study Design

The trial was a randomized parallel and double-blind (patient and evaluator) clinical trial. Advertising was held to invite participation in the dental school and through social networks such as Facebook and Twitter. This randomized clinical study was approved by the Ethics Committee of the Faculty of Dentistry of the University of Chile (2016/04) and was performed according to the Consolidated Standards of Reporting Trials Statement[22] and the Declaration of Helsinki[23] (1975; revised 2000) (Figure 1).

Sample Size

To determine the size of the sample, GPower 3.1[24] software was used, considering a significance level of 5% statistical power of 80% and 10% abandoned based on change of color. This study corresponds to a therapeutic equivalence type, where a color variation of ΔSGU tones in the range of 7 to 10 or more based on the original color was considered significant. This gives a sample size of 20, and to compensate for a potential dropout rate, we used a sample size of 25 per group. In the absence of previous studies that valued biomarkers using this methodology, it was not possible to make a sample calculation based on the values of biomarkers.

Inclusion and Exclusion Criteria

Patients included were over 18 years of age with one or more nonvital teeth. Any restoration did not cover the vestibular face of the tooth, and endodontic treatments were required to be in good condition and without apical lesions, with no previous experience of tooth bleaching, and with a tooth shade C2 or greater according to the Vita Classical scale.

Patients were excluded if they were pregnant or lactating, had enamel hypoplasia, had teeth stained by tetracycline or fluorosis, were in orthodontic treatment with fixed appliances, or had periodontal disease. Also, patients with systemic pathologies were excluded, as were those volunteers who presented clinically and radiographically for with external or internal dental resorption, caries, or periapical lesions. They were informed and referred to specialists for treatment.

Randomization and Allocation Concealment

The teeth (n=50) were randomly divided into two groups according to the bleaching gel to be applied: 25 patients with 35% hydrogen peroxide (Opalescence Endo, Ultradent, South Jordan, UT, USA) and 25 patients with 37% carbamide peroxide (Whiteness Super-Endo, FGM, Joinville, Brazil). A third person who was not involved in the research protocol performed the randomization procedure by using computer-generated tables. We used simple randomization with an equal allocation ratio (www.sealedenvelope.com). Opaque, sealed, and consecutively numbered envelopes containing the identification of the groups were opened immediately only before the beginning of the bleaching protocol.

Study Intervention

The operator was not blinded to the procedure, as both bleaching techniques had different commercial presentations. However, the participants and the examiners who evaluated the color changes were not aware of the allocation of the participants within the study groups. One week before the bleaching therapy commenced, a protective isolating cervical base was applied. For this purpose, the root canal of each tooth was properly isolated with a rubber dam (Rubber Dam Ash, Dentsply, Petropolis, Brazil). Afterward, a final seal with 2 mm of resin-modified glass ionomer cement (Riva Light Cure, SDI, Bayswater, Australia) was placed and light cured for 40 seconds (Cal Radii, SDI). The location 2 mm above the cemento-enamel junction was sealed because of the italic "S" arrangement of the dentinal tubules in this zone; the seal ensured that the cervical crown portions were bleached effectively. The radiographic control was made to corroborate the final seal. In total, three radiographic controls were performed: precontrol, unsealing control, and cervical sealing control.

Bleaching agents were applied according to the manufacturer's instructions in four sessions with an outpatient technique (ie, walking bleaching); each session was separated by one week.[1] In each session, according to the walking bleaching technique, bleach gel was left in the pulp chamber. Subsequently, the cavity was sealed with a temporary cement (Fermin, Detax, Ettlingen, Germany) until the next session. After four weeks of bleaching, the temporary

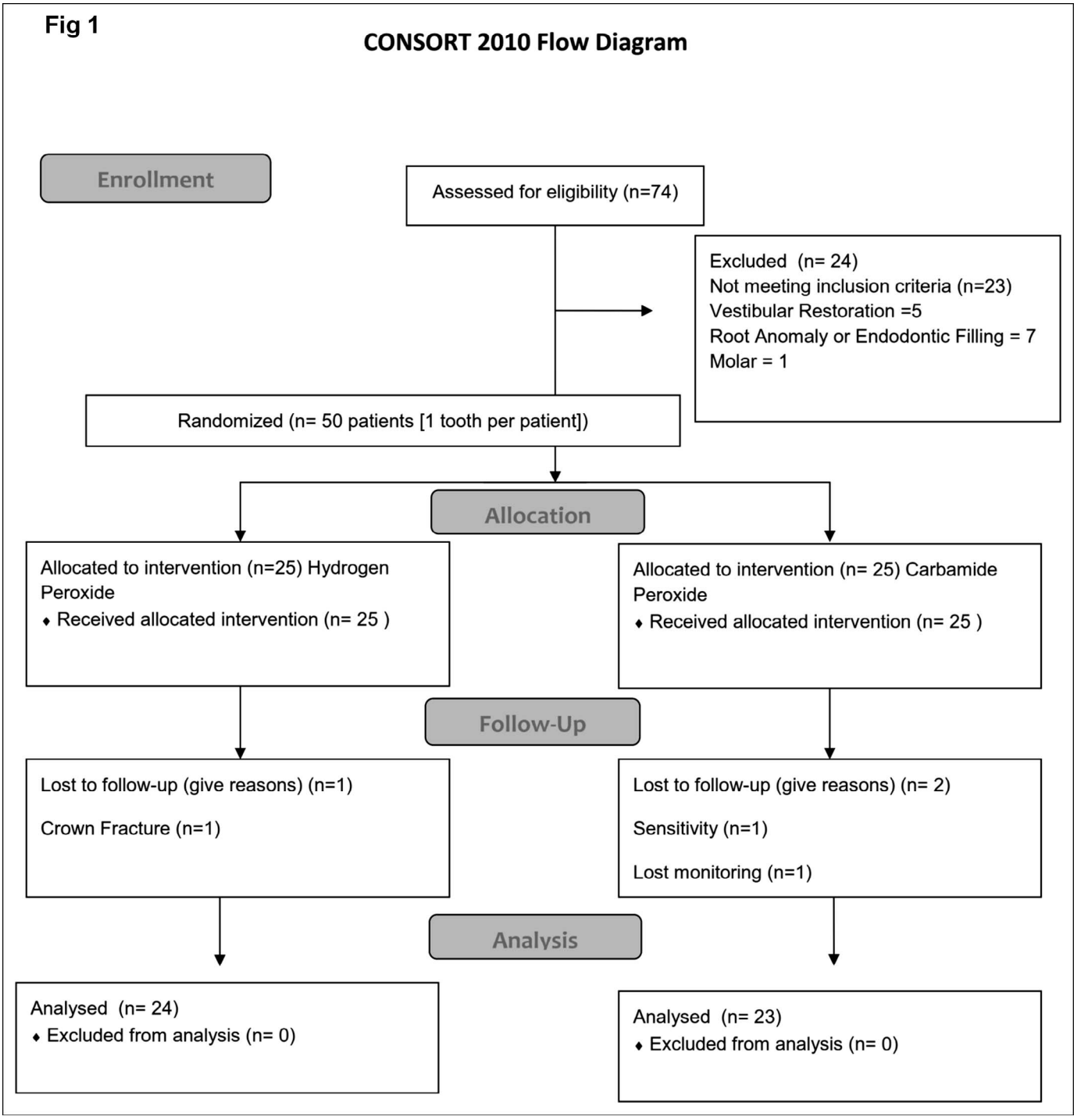

Figure 1. *Consort Flow diagram.*

material was removed, the pulp chamber was washed with water, and a temporary sealing was left for seven days prior to the completion of the final restoration. Patients were advised not to eat or drink foods that might stain their teeth (e.g., coffee, tea, or red wine) during the study period. They were given written instructions and contact information if any questions or problems were to arise.

Color Evaluation

Two calibrated evaluators with 80% agreement (kappa test) recorded the color of teeth at baseline,

Table 1: *Baseline Features of the Participants*

Baseline Features	Groups	
	Hydrogen Peroxide	Carbamide Peroxide
Age (y; means±standard deviation)	30.6 ± 11.7	30.8 ± 11.3
Minimum age (y)	19	20
Maximum age (y)	65	65
Male (%)	47.83	39.13
Trauma (%)[a]	56.52	39.13
Caries (%)[a]	43.48	60.87
VITA Bleachedguide 3D-MASTER median (minimum; maximum)	12 (7; 15)	11 (9; 15)

[a] *Cause of endodontic treatment.*

immediately after each bleaching session, and one week and one month posttreatment. The color evaluation was conducted in the middle third of the labial surface of the tooth bleaching according to the recommendations of the American Dental Association.[25] Patients were examined in the same room with the same lighting, for both examiners independently, using the VITA Bleachedguide 3D-MASTER (Vita Zahnfabrik, Bad Säckingen, Germany). For every "value," a color is assigned a numerical value in order to calculate the change in unit scale (ΔSGU). Color changes were recorded as the difference between the baseline and the different evaluation times, expressed in the number of color guide units (ΔSGU). If the results of the two evaluators did not match, the two evaluators then discussed the case until a consensus was reached concerning the color. The color of the counterpart tooth was also recorded subjectively and compared to that of the treated tooth.

GCF Sample Collection

After isolating the tooth with a cotton roll, supragingival plaque was removed with a curette (Gracey 3/4) without touching the marginal gingiva. The crevicular site was then dried gently with an air syringe, and gingival crevicular fluid (GCF) was collected using absorbent paper strips (Periopaper, OraFlow Inc, New York, NY, USA) that were placed into the selected periodontal sulci until mild resistance was felt and left in place for 30 seconds. Strips contaminated by saliva or blood were excluded. GCF samples were obtained from six periodontal locations: three vestibular and three palatal (mesial, middle, and distal) sites from teeth that underwent the bleaching procedures. Samples were taken before the bleaching (baseline) and after each bleaching session as well as one week and one month postbleaching. Following GCF collection, strips were placed in Eppendorf vials containing 100 μL of phosphate-buffered saline with 0.05% Tween-20 (Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and centrifuged at 10,000 xg for five minutes at 4°C. The elution procedure was repeated twice, and obtained samples were stored at −80°C until further analysis.

Quantification of RANK-L and IL-1β

Total proteins were quantified using Bradford procedures, and then the RANK-L and IL-1β levels were measured using Quantikine ELISA (R&D Systems Inc, Minneapolis, MN, USA) following the manufacturer's instructions. The ELISA kits used in this study were the RANKL ELISA (catalog number 04-BI-20462, Alpco, Biomedica Medizinprodukte GmbH & Co, Vienna, Austria) and the IL-1β ELISA (Human CXCL8/IL-8, D8000C, R&D Systems Inc). Absorbance was measured at 492 nm with a wavelength correction of 630 nm using an automatic microplate reader (Synergy HT, Bio-Tek Instrument Inc, Winooski, VT, USA). The concentration of each marker in each sample was calculated by a four-parameter logistic equation.

Statistical Analysis

Statistical analysis was performed using SPSS 23.0 (SPSS Inc, Chicago, IL, USA) with $\alpha = 0.05$. For intragroup analysis, the Wilcoxon test was used; for intergroup analysis, we used the Mann-Whitney test.

RESULTS

A total of 74 volunteers were examined, and 50 patients met the inclusion criteria. Fifty teeth were selected, one from each patient, of which three did not complete the treatment, and one tooth was excluded from analysis due to problems with the analysis of samples of the gingival fluid. The final

Table 2: *Changes of Color by ΔSGU (VITA Bleachedguide 3D-MASTER) by Group in Different Time Frames Expressed by Median, Minimum, Maximum, and Statistical Significance (Minimum; Maximum)*[a]

Assessment Points	Color Change		Mann-Whitney Between Groups
	Hydrogen Peroxide	Carbamide Peroxide	
Baseline vs 1 wk of bleaching	3 (0; 5) A	1 (0; 4) A	0.410
Baseline vs 2 wk of bleaching	4 (0; 8) A	2 (0; 5) A	0.002
Baseline vs 3 wk of bleaching	5 (0; 9)	4 (1; 5) A	0.007
Baseline vs 4 wk of bleaching	5.5 (0; 9)	4 (2; 8)	0.019
Baseline vs 1 wk after bleaching (before restoration)	5 (0; 9)	4 (2; 7)	0.037
Baseline vs 1 wk after bleaching (after restoration)	5 (0; 9)	4 (2; 7)	0.040
Baseline vs 1 mo after bleaching	5 (0; 9)	4 (2; 7)	0.027

[a] *Mann-Whitney test was applied only for pairwise comparison between groups at each time ($\alpha=0.05$). Wilcoxon rank statistical test was applied only for comparison of different times for each group ($\alpha=0.05$). Values with the same letter indicate statistically significant difference with all times in the group ($p<0.05$).*

sample consisted of a total of 46 nonvital teeth that were bleached; the distribution of the group can be seen in Table 1.

Color Evaluation

The color change results are shown in Table 2. Hydrogen peroxide showed a high degree of whitening after the third session of bleaching to the one-month follow-up ($p<0.05$) compared with the carbamide-peroxide group, which achieved a significant difference of color change after the fourth session ($p<0.05$).There was a statistically significant difference ($p<0.05$) in the third and fourth weeks, showing more color change in the hydrogen peroxide group. At one week postbleaching, the color change did not present significant differences. Significant whitening was observed in both study groups after four weeks of treatment ($p<0.05$). The hydrogen peroxide group showed a higher degree of whitening in the second, third, and fourth weeks of bleaching when compared to the carbamide peroxide group ($p<0.02$).There was a difference between the degree of whitening between groups at one month post-bleaching ($p<0.03$).

Quantification of RANK-L

In total, 966 samples were taken from each group for quantification of RANK-L and IL-1β. Figure 2 shows the levels of RANK-L expressed in pg/μL, considering all the sites. All evaluation times were increased with statistically significant differences between them ($p<0.05$). Table 3 shows the RANK-L levels expressed in differences between the baseline and time values (delta). The comparison of delta values between groups showed no statistically significant differences at the times assessed ($p>0.075$).

Quantification of IL-1β

Figure 3 shows the levels of IL-1β expressed in pg/μL, considering all the sites. All evaluation times have statistically significant differences between them ($p<0.05$). Concerning the total values of IL-1β, there was a statistically significant difference at three weeks of bleaching ($p<0.05$) between the groups; however, there was no significant difference for the total values by vestibular or palatal sites at the same time between the groups ($p>0.1$). Table 3 shows the IL-1β levels expressed in differences between the baseline and time values (delta). The comparison of delta values between groups showed no statistical difference at the times assessed ($p>0.06$) except at the three-week session ($p=0.049$).

DISCUSSION

The results of the present investigation showed that both products showed a significant color change after four weeks of treatment, although hydrogen peroxide exhibited a faster effect. This may be due to the slower chemical decomposition of carbamide peroxide and its lower penetration into dental tissues compared to hydrogen peroxide;[16,26,27] ie, carbamide peroxide has a chemical decomposition rate that is one-third that hydrogen peroxide. Both products showed increased levels of similar markers (Tables 3 and 4). One explanation for this is that the concentration of hydrogen peroxide released from gel carbamide peroxide (16%) may be sufficient to aggravate periodontal tissues.

The most important topic to be presented in this study is whether hydrogen peroxide or carbamide peroxide could be responsible for increasing the levels of RANK-L and IL-1β. The results of the present trial suggest that hydrogen peroxide and carbamide perox-

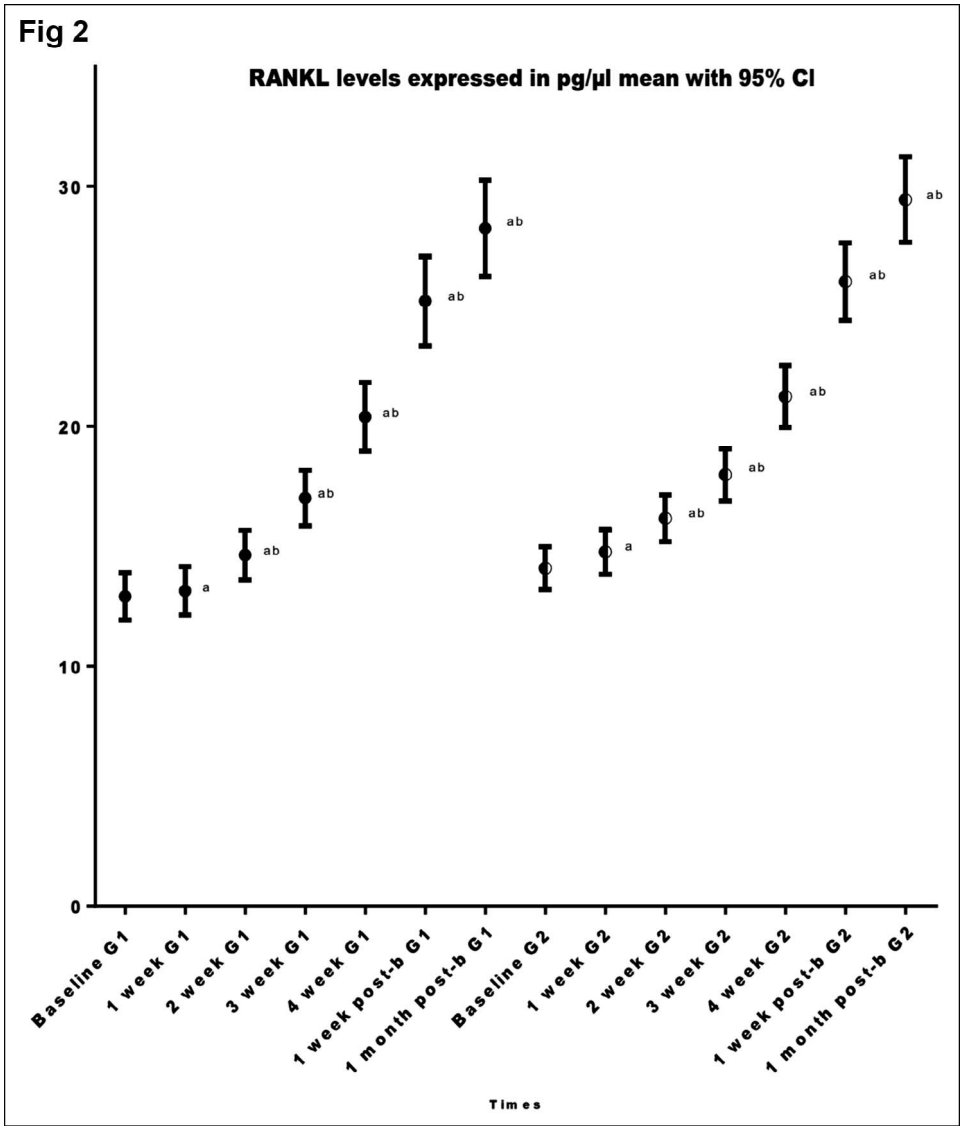

Figure 2. *RANK-L levels at times expressed in pg/µL, mean with 95%. a, statistically significant difference with baseline with the Wilcoxon test ($p<0.05$); b, statistically significant difference with previous time with the Wilcoxon test ($p<0.05$).*

ide influence the RANK-L-RANK–OPG axis by significantly increasing RANK-L and IL-1β from the first session of nonvital bleaching until one month postbleaching. This study showed a constant increase of biomarkers even one month postbleaching (Tables 3 and 4); therefore, the question remains, When does the increase of biomarkers stop? The answer is that hydrogen peroxide triggers a chronic process into the periodontium and can exhibit a duration over one month postbleach–gel contact.

This indicates that the significant increase of levels of markers of bone destruction (ie, RANK-L and IL-1β) could be responsible for the phenomenon of external root resorption, a pathology associated with the walking bleaching technique when applied in endodontically treated teeth.[28-30]

IL-1β is a potent osteoclast activity initiator[31] and generally stimulates macrophages, monocytes, or endothelial cells, among others, to produce metalloproteinases, prostaglandins, and other proinflammatory cytokines.[32,33] Also, it stimulates RANK-L production of osteoblasts, causing differentiation and maintenance of osteoclasts.[34] Among the factors that trigger increased IL-1β are nonmicrobial factors, such as tissue injury or the presence of inflammatory molecules, among others;[32] this situation is initiated by the peroxide released from the pulp chamber to the periodontium.

The role of IL-1β, a key cytokine with proinflammatory functions, has not yet been studied in the case of bleaching treatment. Studies have linked increased levels of IL-1β in teeth with apical external resorption associated with orthodontic treatment

Table 3: *Delta IL-1β and RANKL Levels Expressed in pg/μL, Median (Minimum; Maximum)*[a]

Assessment Points	Delta IL-1β		Delta RANK-L	
	ΔHP	ΔCP	ΔPH	ΔPC
1 wk of bleaching vs baseline	2.77 (−1.69; 17.32)	2.64 (−1.91; 30.03)	0.52 (−2.61; 2.80)	0.54 (−0.88; 4.77)
2 wk of bleaching vs baseline	9.78 A (0.77; 35.36)	8.64 A (0.30; 57.92)	1.92 A (−0.88; 6.57)	1.71 A (−0.70; 9.24)
3 wk of bleaching vs baseline	21.53 A (2.75; 73.01)	18.27 A (0.49; 107.03)	4.17 A (-0.47; 13.81)	3.40 A (0.18; 13.19)
4 wk of bleaching vs baseline	40.38 A (7.86; 147.22)	34.23 A (3.12; 177.58)	8.02 A (0.30; 22.76)	6.62 A (1.72; 18.19)
1 wk after bleaching vs baseline	66.98 A (13.10; 201.01)	62.62 A (9.91; 222.92)	12.17 A (1.91; 37.67)	11.19 A (2.61; 29.83)
1 mo after bleaching vs baseline	81.74 A (21.79; 237.21)	79.37 A (16.47; 254.03)	15.20 A (0.80; 46.45)	14.57 A (4.18; 36.41)

Abbreviations: Δ, assessment points vs baseline; HP, hydrogen peroxide; CP, carbamide peroxide.
[a] *Wilcoxon rank statistical test was applied only for comparison of different times for each group ($\alpha=0.05$). Values with the same letter indicate statistically significant difference with previous time with the Wilcoxon test ($p<0.05$).*

sequelae.[35] This demonstrates the presence of an inflammatory process,[36] which can explain the unbalanced levels of RANK-L.[37] Royaka and others, in a recent study, explain that extraradicular diffusion during intracoronal bleaching with different bleaching agents.[27]

Also, the imbalance shift of IL-1β and RANK-L levels is a finding that could be explained initially by the sudden drop in pH and, in turn, autocatalyzation to achieve a stabilization of metalloproteinase and cathepsin. This is because, according to a recent report, proteolytic enzymes, cysteine cathepsins, and matrix metalloproteinases are activated in mineralized dentin during degradation from tooth-bleaching treatment with 35% H_2O_2.[12]

Although no clinical or radiographic signs of external root resorption were observed in the present study—mainly because this is a chronic process—the findings of the present study may explain to some extent the beginning of this process, mainly because there is an increase in markers responsible for the activation of osteoclasts. This may be a predisposing factor for the marginal bone resorption and an explanation for the phenomenon of external cervical resorption–mediated odontoclasts, which is still not explained in the literature.

It is worth mentioning that the increased levels of RANK-L are not comparable to those achieved by a patient with active periodontal disease since these levels could suggest an activation process of bone resorption in patients with high periodontal risk.[38] However, increasing the levels of IL-1β (about 190 pg/μL) is comparable with active sites of periodontitis,[39] indicating that this damage is relevant, mainly because after completion of bleaching and up to one month of monitoring, there is a continuous and progressive increase in the levels of IL-1β and RANK-L.

It is important to mention that a high variability of marker levels was found during all phases of the study; also, some significant differences were found for IL-1β when palatal and vestibular sites were compared in the same tooth. This could be explained by several factors. The anatomy of endodontically treated teeth and the thickness of the remaining walls are not uniform, so it is expected that in teeth with less dentin thickness, diffusion of peroxide into the periodontal regions can have a greater effect.[40] In addition, the biological response of each individual is different.[39] Perhaps some cracks and fractures associated with traumatic injuries could be responsible for the faster spread of peroxide. Also, the inflammatory response with high intersubject variability may better explain root resorption mediated by increased levels of IL-1β and RANK-L markers.

The technique evaluated in the present study applied the bleaching products inside the pulp chamber to facilitate contact with dentinal tissue and to maintain that contact for a long period of time (one week)[1,41,42] during which the chemical agent makes prolonged contact with the tooth; this favors the spread of peroxide into the extraradicular space, which would cause an increase in the markers studied. This study coincides with that of Firat,[43] where an in-office bleaching gel was applied in vital teeth without direct contact with the dentinal tissue and showed that an increase in levels of IL-1β was produced by direct contact of three gels with higher concentrations of hydrogen peroxide (>35%) despite the fact that this amount of

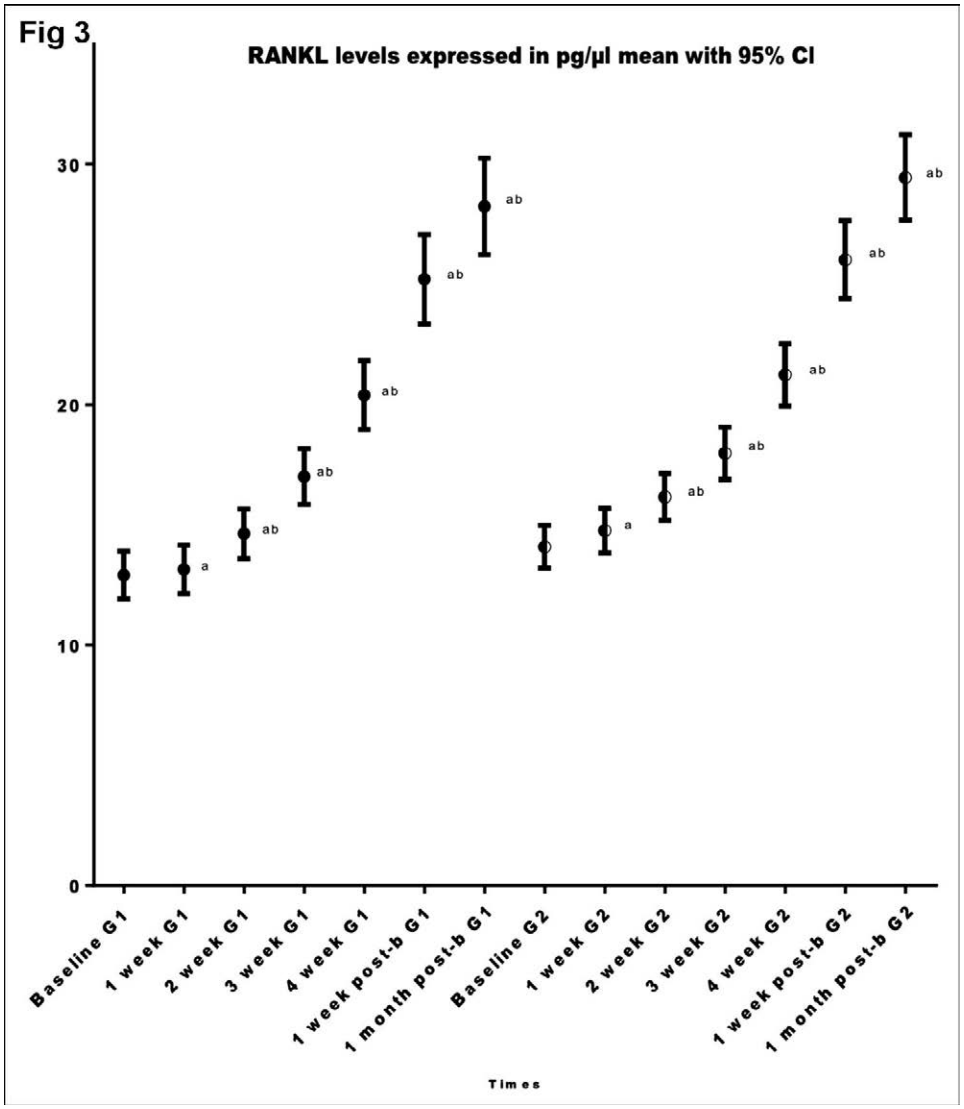

Figure 3. *IL-1β levels at times expressed in pg/µL , mean with 95%. a, statistically significant difference with baseline with the Wilcoxon test (p<0.05); b, statistically significant difference with previous time with the Wilcoxon test (p<0.05).*

IL-1β is very low (<73 pg/µL per site) compared with the results of this study (Table 3). This reinforces the importance of remaining hydrogen peroxide in the pulp chamber.

Future studies should use in-office bleaching only on the enamel surface of nonvital teeth to evaluate bone markers, such as RANK-L and IL-1β.

CONCLUSIONS

The walking bleaching technique with 35% hydrogen peroxide and 37% carbamide peroxide results in constantly increasing levels of RANK-L and IL-1β in the gingival crevicular fluid around the bleached teeth through one month postbleaching; this is associated with a chronic process within the periodontium apparently of unknown behavior—that is, the biomarkers increased until the one-month follow-up, and we do not know when they will return to normal. The change of color in the hydrogen peroxide group was faster than the carbamide peroxide group when using a walking bleaching technique and when measured subjectively, but both groups showed high effectiveness.

Acknowledgement

The work was supported by Fondecyt 1140904.
The author dedicates this article to her two kids Elisa and Eduardo by their enormous inspiration.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Comité Etico-Científico de la Facultad de Odontología de la Universidad de Chile (FOUCH). The approval code for this study is 2016/04.

Conflict of Interest

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

(Accepted 28 April 2017)

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Five-year Clinical Evaluation of a Nanofilled and a Nanohybrid Composite in Class IV Cavities

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Clinical relevance

Nanofilled and nanohybrid composites resulted in a similar clinical performance in class IV cavities. Restoration fracture was the most important cause of failure.

SUMMARY

The purpose of this study was to evaluate a nanofilled and a nanohybrid composite, in combination with manufacturer-recommended etch-and-rinse adhesives, in class IV cavities. Thirty-four patients aged 14-46 years (mean age, 27.1 years) comprised the study group. Twenty-six patients received two class IV restorations and eight patients received four class IV restorations. For each patient, half the number of restorations were performed using a nanohybrid composite (Ceram X duo) and the remaining half used a nanofilled resin composite (Filtek Supreme XT), with two- (XP Bond) and three-step (Scotchbond Multipurpose) etch-and-rinse adhesives, respectively. Two experienced examiners evaluated the restorations for retention, color match, marginal discoloration, wear/loss of anatomic form, caries formation, marginal adaptation, and surface texture to compare the baseline (after placement) and annual recalls over 5 years. The cumulative success rates for the Filtek Supreme XT and Ceram X duo restorations after five years were 86.2% and 89.7%, respectively. Four Filtek Supreme XT and three Ceram X duo restorations failed. There was no statistically significant difference between the nanofilled and nanohybrid composites at any of the evaluation periods for any of the parameters evaluated. Despite the limited number of restorations, all restorations were clinically acceptable regarding retention, color match, marginal discoloration, wear or loss of anatomic form, the formation of caries, marginal adaptation, and surface texture, except the failed restorations. Fracture was the main cause of restoration failure.

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DOI: 10.2341/16-358-C

INTRODUCTION

The increasing demand for esthetic treatment of anterior teeth indicates that the causes of restoration failure, beyond caries and fracture, occur largely in anterior restorations. However, long-term clinical trials are required to determine the validity of this supposition. Another important consideration is whether aspects of the composite formulation used, such as filler size, affects the clinical performance of anterior restorations.[1]

Various composites are available for anterior restorations.[1] In anterior teeth, the esthetic appearance, color matching, and polishability are important. Anterior composites usually have small filler particles to increase smoothness, but this also reduces the fracture strength and Young's modulus of the materials.[2] However, nanotechnology has enabled recent advances in dental composite restorations.[3] Consequently, a new category of composite resins, known as nanocomposites, has been developed.[3,4] Although all filler particles should be nanometer-sized in true nanocomposites, the term "nanotechnology" is occasionally used inappropriately when describing materials. At present, two distinct dental composites, nanofillers and nanohybrids, contain nanoparticles. Nanofilled composites use nanoparticles (1-100 nm) throughout the resin matrix, whereas nanohybrids contain conventional fillers (0.4-5.0 μm), with nanometric particles.[3] Increasing the filler load by adding these particles improves the mechanical properties and polishability of the composite surfaces.[5]

Several nanocomposite products are available on the market.[6] A new nanofilled composite was recently developed that contains nanometric particles (combination of 20 nm nonagglomerated/nonaggregated nanosilica) and nanoclusters (primarily 2- to 20-nm loosely bound zirconia/silica particle agglomerates).[6,7] Another nanocomposite is the ormocer-based nanoceramic composite, Ceram X (Dentsply-DeTrey, Konstanz, Germany), which contains a methacrylate-modified silicon dioxide-containing nanofiller (10 nm) and glass fillers (1.1-1.5 μm); furthermore, the conventional resin matrix is largely replaced by a matrix replete with highly dispersed methacrylate-modified polysiloxane particles (2-3 nm).[6] The various esthetic composite system formulations also include a vast range of shade options for dentin and enamel layers, as well as several areas of the teeth, such as the incisal edge, body, and cervical. Thus, clinicians are able to choose a shade-layering technique, according to the esthetic needs of the patients.[3]

In contrast to class I, II, III, and V cavity configurations, stresses are generated in class IV restorations through the incisal angle, which challenges the tooth-restoration interface.[8] There are also greater challenges to the tooth-restoration bonded interface because of the lack of mechanical retention in the majority of class IV restorations.[1] The etch-and-rinse method remains the most effective approach to achieving efficient and stable bonding to enamel.[9] Also, etch-and-rinse adhesives, which result in higher resin-dentin bonds, are more durable than most one- and two-step dentin adhesives.[10]

To date, no published studies have evaluated the longevity of highly stressed class IV nanofilled and nanohybrid restorations. Therefore, we aimed to evaluate a nanofilled and a nanohybrid composite, in combination with their manufacturer-recommended etch-and-rinse adhesives, in class IV cavities.

METHODS AND MATERIALS

Study Design

The brands, chemical compositions, and manufacturers of the materials used are shown in Table 1. The restorations were placed between August 2008 and February 2010 at the Department of Restorative Dentistry, Faculty of Dentistry, Istanbul University. In total, 34 patients (9 males and 25 females) aged between 14 and 46 years (mean age, 27.1 years) comprised the study group. Table 2 lists the inclusion and exclusion criteria for the patients.[11-14] Twenty-six patients received two class IV restorations and eight patients received four class IV restorations, due to primary caries of the anterior teeth. A total of 84 restorations were included in the final analysis. The distribution of class IV restorations, according to adhesive/composite combination and tooth number, is presented in Table 3. Opposing and adjacent tooth contacts were present for all teeth.[14]

Treatment Protocol

For the 26 patients who received two class IV restorations, one was performed with a nanofilled resin composite (Filtek Supreme XT, 3M ESPE, St Paul, MN, USA), whereas the other was performed with a nanohybrid composite (Ceram X duo). Similarly, for the eight patients who received four class IV restorations, two were performed with Filtek Supreme XT, whereas the other two were performed with Ceram X duo. The nanohybrid and nanofilled resin composites were

Table 1: *Materials, compositions, and application steps*

Adhesive	Components	Application Steps	Manufacturer
Scotchbond Multi Purpose	Etchant: Scotchbond Etchant (35% H_3PO_4) Primer: 2-hydroxyethylmethacrylate, polyalkenoic acid, copolymer, water Adhesive: 2-hydroxyethylmethacrylate, bis-GMA, photoinitiator	Apply the etchant for 15 seconds. Rinse the surface for 15 seconds and dry the surface slightly leaving a visible moist surface. Apply the primer and dry gently for five seconds. Apply the adhesive and light cure for 20 seconds.	3M ESPE
XP Bond	Etchant: DeTrey Conditioner 36 (36% H_3PO_4) Adhesive: PENTA, TCB, UDMA. TEGDMA. HEMA, Camphorquinone, DMABE, Butylated benzenediol, tert-Butanol, Functionalized amorphous silica	Apply conditioner to the dentin surface for 15 seconds. Rinse thoroughly for 15 seconds and Remove rinsing water completely by blowing gently with an air syringe. Dentin did not desiccated. Apply adhesive to wet all the tooth surfaces uniformly and leave undisturbed for 20 seconds. Airblow for at least five seconds. Light cure for 20 seconds	Dentsply DeTrey
Filtek Supreme XT	Bis-GMA, UDMA, TEGDMA, bis-EMA, filler load 59 vol.% (78.5 wt.%). Filler particles: combination of a nonagglomerated/nonaggregated, 20-nm nanosilica filler, and loosely bound agglomerated zirconia/silica nanocluster, consisting of agglomerates of primary zirconia/silica particles with size of 5- to 20-nm fillers. The cluster particle size range is 0.6 to 1.4 μm	Tooth color to be restored selected from a Vitapan classic shade guide before isolating the tooth. The corresponding body shade is selected. Increments of body shade applied in 2-mm layers or less. Each increment light cured 20 seconds.	3M ESPE
Ceram X duo	Methacrylate modified polysiloxane, dimethacrylate resin, fluorescence pigment UV stabilizer, camphorquinone, ethyl-4(dimethylamino)benzoate, barium-aluminium-borosilicate glass, methacrylate functionalized silicon dioxide nano filler, iron oxide pigments and titanium oxide pigments and aluminium sulfo silicate pigments	Tooth color to be restored selected from a Vitapan classic shade guide before isolating the tooth. The corresponding combination of Ceram X duo enamel and dentin shade is selected. In 2-mm layers or less dentin shade applied and light cured 40 seconds. Then enamel layer in 2 mm layers or less applied and light cured 10 seconds.	Dentsply DeTrey, Konstanz

Abbreviations: BIS-EMA, ethoxylated bisphenol A glycol dimethacrylate; Bis-GMA, bisphenol A diglycidyl methacrylate; DMABE, ethyl-4-(dimethylamino)benzoate; H3PO4, phosphoric acid; HEMA, 2-hydroxyethyl methacrylate; PENTA, dipentaerythritol pentaacrylate monophophate; UDMA, urethane dimethacrylate; TCB, butan-1,2,3,4-tetracarboyxlic di-2-hydroxyethylmethacrylate ester; TEGDMA, triethylene glycol dimethacrylate.

applied in combination with two-step (XP Bond, Dentsply-DeTrey) and three-step (Scotchbond Multipurpose, 3M ESPE) etch-and-rinse adhesives, respectively (Table 1). The nanohybrid composite and tooth number were randomly selected by flipping a coin. This approach was first used for the patients with two restorations, and the same randomized approach was then used to select the nanofilled and nanohybrid resin composite and tooth number, respectively, for the patients with four restorations. After randomization, the number of restorations per patient with Filtek Supreme XT was equal to the number of Ceram X duo restorations.

Restoration Procedure

The teeth were first cleaned using a pumice-water slurry and a rubber cup to remove the pellicle and any residual dental plaque. All material was used in accordance with the manufacturer's instructions (Table 1). Cavity preparation was limited to the removal of caries. The cavity margins included the proximal area and the incisal surface, as well as the extended facial and lingual surfaces, depending on the amount of tooth structure missing. All enamel margins were beveled at a 45° angle to the external cavosurface, using a high-speed, water-cooled, rotary handpiece, with a medium-grit diamond bur. The width of the bevel was approximately 0.5-2.0 mm,

Table 2: *Inclusion and exclusion criteria*

Inclusion criteria
Receiving at least two primary caries on proximal surfaces of anterior teeth that involve the incisal surface
Having no active periodontal or pulpal diseases
Willing to return for follow-up examinations as outlined by the investigators
Exclusion criteria
Patients with uncontrolled parafunction
Patients presenting insufficient oral hygiene
Patients with spontaneous pain or sensitivity to percussion
Patients were pregnant or nursing
Patients had periodontal or gingival disease

depending on the amount of tooth structure missing and the retention perceived necessary.[15] After the cavities were prepared, the cavity treatment and restorative placement were performed with strict attention to the manufacturer's instructions. Isolation was achieved with cotton rolls and saliva ejectors.[16] Cavity treatment, application, and polymerization of the dentin adhesives were performed by the same experienced practitioner (HSS), who was familiar with the materials being used in the study. After the shade selection, all cavities were restored using a Mylar strip and wooden wedge to rebuild the anatomic form and proximal teeth contacts. The composite was incrementally applied, when the restorations had depths of more than 2 mm. The first composite layer was applied on the pulpal walls and light-cured (Table 1). A second layer was then applied and light-cured, as per the first layer.[16] Care was taken when closing the strip, to not pull with excessive force because the soft material could be extruded incisally, resulting in an under-contoured restoration. In this instance, composite was added to achieve the appropriate contour and contact.[15] Light-curing, contouring, and finishing were performed, as per our previous publication.[14]

Table 3: *Distribution of class IV composite restorations according to adhesives/composite combination and tooth number*

Dentin Adhesive/Composite	n	Tooth No					
		6	7	8	9	10	11
Filtek Supreme XT/Scotchbond Multipurpose	42	1	7	17	14	3	0
ceram.X Duo/XP Bond	42	1	11	12	9	9	0
Sum of restorations	84	2	18	29	23	12	0

Evaluation

Two experienced examiners evaluated the restorations, using a dental explorer and mirror, according

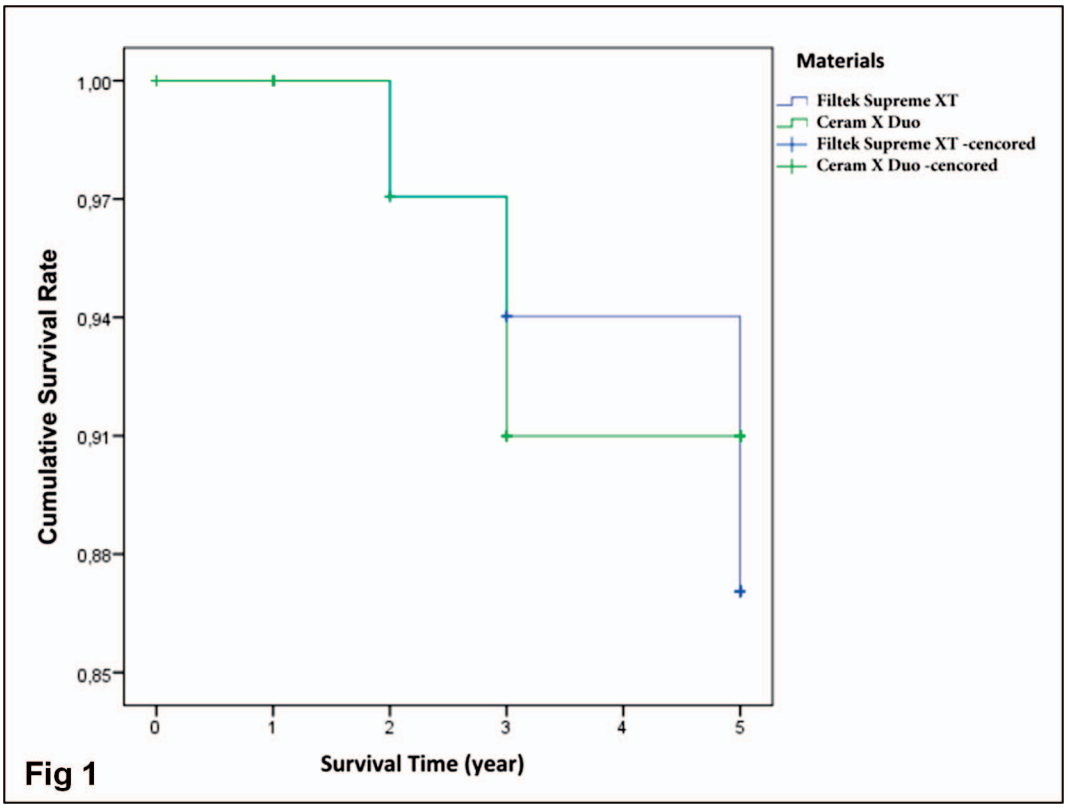

Figure 1. *Kaplan-Meier survival analysis, showing the time interval (years).*

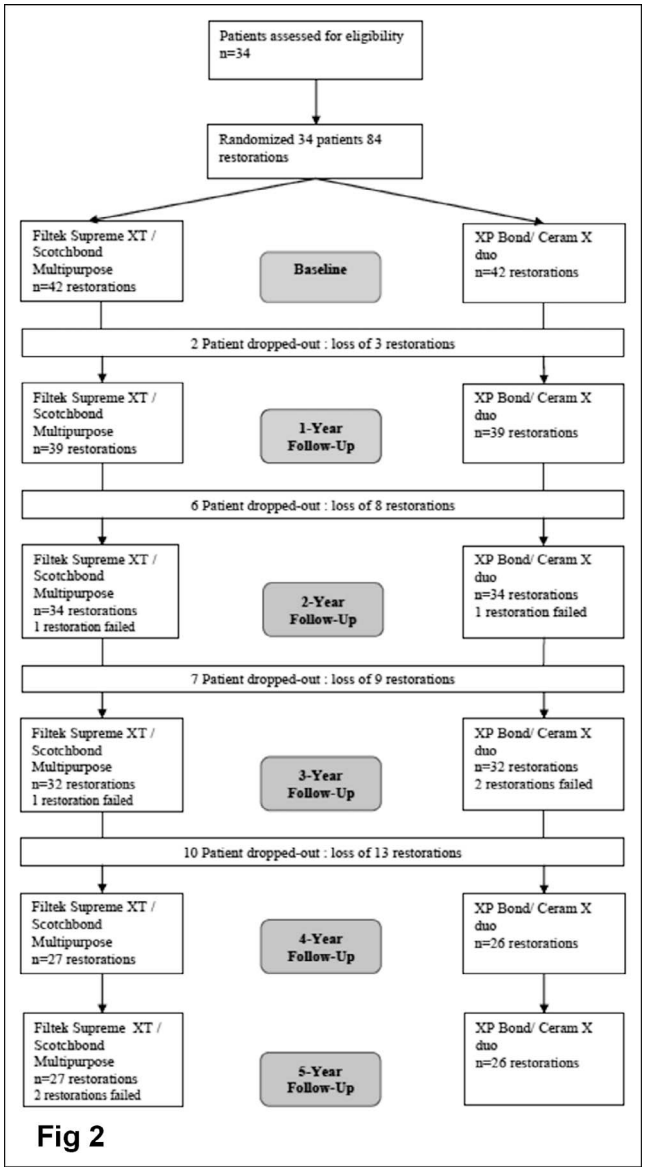

Figure 2. *Flow diagram history of restorations.*

to the modified United States Public Health Service (USPHS) criteria (Table 4).[17-19] The examiners were not involved in the operation and insertion of the restoration procedure and were fully blinded to the experimental protocol. For consistency, both examiners observed sets of photographs as reference material to illustrate the scoring for each criterion. Both examiners then clinically evaluated 20 class IV restorations, which were not included in the study, with a two-day separation between the examinations. The evaluation phase of the study began after obtaining at least 85% intra- and inter-examiner agreement in the calibration phase.[20] Color match, wear or loss of anatomic form, marginal discoloration, caries, marginal adaptation, and surface texture were evaluated at baseline (after placement) and at five annual recalls. Restorations were scored as follows: alpha = ideal clinical condition; bravo = clinically acceptable; charlie = unacceptable condition, restoration requires replacement; and delta = restoration fractured, mobile, or missing, immediate replacement required. Conflicts in scoring were resolved through consensus.[17,20]

Statistical Analysis

All analyses were performed using SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). Data were statistically analyzed using Friedman's test, to examine changes that occurred during the five-year evaluation period (Table 5). Whenever a statistically significant difference was identified, Dunn's test was used for multiple comparisons between each recall time interval for each composite. The Mann-Whitney test was used to evaluate the differences between the two composite materials. The probability of clinical survivability of the two composite types was determined using Kaplan-Meier survival analysis (Figure 1). $p<0.05$ was considered statistically significant. Inter- and intra-examiner agreements were tested using Cohen's κ.

RESULTS

Ten patients, which accounted for 26 restorations, exited from the study during the five-year evaluation (13 Filtek Supreme XT and 13 Ceram X duo restorations; Figure 2). Cumulative recall rates at baseline and at all five annual follow-ups are provided in Table 5. Cohen's κ (0.89) demonstrated strong agreement between the examiners, and there was no statistical difference between their findings ($p>0.05$). The cumulative failure and success rates, according to Kaplan-Meier survival analysis, are shown in Table 5. At two years, one Filtek Supreme XT had failed due to postoperative sensitivity (pulpitis) and one Ceram X duo restoration had failed due to fracture. The success rate was 97.1% for both restorations. At three years, one Filtek Supreme XT and two Ceram X duo restorations had failed because of fracture, with cumulative success rates of 93.9% and 90.9%, respectively. There were no restoration failures at four years, thus providing cumulative restoration success rates of 93.1% for Filtek Supreme XT and 89.7% for Ceram X duo. At five years, one Filtek Supreme XT restoration was lost and one Filtek Supreme XT restoration failed because of fracture. Thus, the cumulative success

Table 4: *Direct clinical evaluation criteria (modified USPHS criteria)*

Rating	Aspect	Method
Color match		
Alpha (A)	There is no mismatch in color, shade and/or translucency between the restoration and the adjacent tooth structure	Visual inspection
Bravo (B)	There is a mismatch in color, shade and/or translucency between the restoration and the adjacent tooth structure, but the mismatch is within the normal range of tooth color, shade, and/or translucency	Visual inspection
Charlie (C)	The mismatch is between restoration and adjacent tooth structure outside the normal range of tooth color, shade and/or translucency	Visual inspection
Cavosurface marginal discoloration		
Alpha (A)	There is no discoloration anywhere on the margin between the restoration and the tooth structure	Visual inspection
Bravo (B)	There is discoloration anywhere on the margin between the restoration and the tooth structure, but the discoloration has not penetrated along the margin of the restorative material in a enamel direction and can be polished away	Visual inspection
Charlie (C)	The discoloration has penetrated along the margin of the restorative material in a enamel direction	Visual inspection
Wear/Anatomic form		
Alpha (A)	The restoration is not under-contoured, that is, the restorative material is not discontinuous with existing anatomic form	Visual inspection and explorer
Bravo (B)	The restoration is under-contoured, that is, the restorative material is discontinuous with existing anatomic form, but sufficient restorative material is not missing to expose the enamel or base	Visual inspection and explorer
Charlie (C)	Sufficient restorative material is missing to expose the enamel or base	Visual inspection
Caries		
Alpha (A)	There is no evidence of caries contiguous with the margin of the restoration.	Visual inspection
Bravo (B)	There is evidence of caries contiguous with the margin of the restoration.	Visual inspection
Marginal adaptation		
Alpha (A)	There is no visible evidence of a crevice along the margin into which the explorer will penetrate.	Visual inspection and explorer
Bravo (B)	There is visible evidence of a crevice along the margin into which the explorer will penetrate. The enamel or base is not exposed.	Visual inspection and explorer
Charlie (C)	There is visible evidence of a crevice along the margin into which the explorer will penetrate. The enamel or base is exposed.	Visual inspection and explorer
Delta (D)	The restoration is fractured or missing in part or *in toto*.	Visual inspection and explorer
Surface texture		
Alpha (A)	Surface of restoration is smooth.	Explorer
Bravo (B)	Surface of restoration is slightly rough or pitted, can be refinished.	Explorer
Charlie (C)	Surface deeply pitted, irregular grooves (not related to anatomy), cannot be refinished.	Explorer
Delta (D)	Surface is fractured or flaking.	Explorer

rates for the Filtek Supreme XT and Ceram X duo restorations were 86.2% and 89.7%, respectively.

Two of four failed Filtek Supreme XT restorations were located in the mesial region of the right central incisor. One restoration was located in the mesial region of the left central incisor and one restoration was located in the mesial region of the right lateral incisor. One of three failed Ceram X duo restorations was placed in the mesial region of the right lateral incisor. One restoration was located in the mesial region of the left lateral incisor, and one restoration was placed in the distal region of the left lateral incisor.

The direct clinical evaluation rates, at baseline and at the five annual recalls, are shown in Table 5. At five years, with the exception of the four failed Filtek Supreme XT and three Ceram X duo restorations, no restorations were clinically unacceptable, regarding any of the evaluation criteria. Moreover, there were no statistically significant differences between Filtek Supreme XT and Ceram X duo restorations, in any of the evaluation periods, for any of the evaluation parameters.

At five years, 92% of Filtek Supreme XT and 84.6% of Ceram X duo restorations exhibited alpha color matches. Regarding the marginal discoloration, 16% of Filtek Supreme XT and 11.5% of Ceram X duo restorations showed bravo discoloration. However, the discoloration was superficial and was located on an unspecific part of the enamel. It did not penetrate

toward the pulp at the edge of the restoration and could be polished away. Regarding wear and anatomic form, 88% of the Filtek Supreme XT and 96.2% of the Ceram X duo restorations were assessed as alpha at five years. Regarding marginal adaptation, three (6.3%) Ceram X duo restorations and two (7.4%) Filtek Supreme XT were regarded as unacceptable (charlie) at three and five years, respectively, and had to be replaced at the end of the five-year period. For caries and surface texture, 100% of both the Filtek Supreme XT and Ceram X duo restorations were graded as alpha at five years.

DISCUSSION

A meta-analysis about the clinical effectiveness of anterior restorations concluded that new clinical research on contemporary resin materials was warranted, owing to the few studies that exist regarding class IV restorations, most of which were performed between 1980 and 2000 with older materials.[21] Incisal edge class IV restorations are exposed to high masticatory loads.[1] Thus, the mechanical and physical properties of a restorative material are as important as its esthetic features. Ideal esthetic restorative materials should simulate natural teeth in color, texture, and translucency and also have adequate strength, wear, and sealing characteristics.[22] Nanocomposite systems were shown to have a high translucency, polish, and polish retention similar to microfilled composites and maintained the physical and wear resistance properties of various hybrid composites.[23] Therefore, the present study evaluated the five-year clinical performance of a nanofilled and a nanohybrid composite, in combination with their manufacturer-recommended etch-and-rinse adhesives, in class IV cavities.

In the current study, the five-year survival rates for Filtek Supreme XT/Scotchbond Multipurpose restorations and Ceram X duo/XP Bond restorations were 86.2% and 89.7%, respectively. In contrast, a relatively long-term clinical study (up to 20 years) found a 100% anterior restoration five-year survival rate, which included class III and IV restorations.[2] The discrepancy between this finding and the current results may be due to differences in the number of restorations (51 vs 84 class IV restorations, respectively) and the material and patient characteristics. Also, in contrast to the present study, which only included one type of cavity (class IV), a 100% success rate was previously reported in class III/IV restorations at five years.[24] This high success rate in composite restoration success in recent years is a result not only of improved restorative materials and bonding systems but of increased clinician expertise. However, teeth having a combination of endodontic access and two-type cavities (class III/IV), as well as a comparably lower number of class IV restorations (16 restorations),[24] may explain the variable success rate. In another study, 4 of 45 class III/IV restorations, with two-step etch-and-rinse adhesive, failed at 48 months.[25] However, in accordance with the present study findings, van Dijken showed a cumulative survival of more than 80% in the Kaplan-Meier survival curve for a composite restorative material (Pekafil) at five years, in a maximum 14-year follow-up of class IV restorations.[22] Also, fractures accounted for 11 of 43 restorations (25.6%), with a Kaplan-Meier estimate of 9.9 years,[22] corroborating our findings that found fracture was the main reason for class IV restoration failure.[22] Furthermore, in agreement with the present study results, a meta-analysis reported 10-year survival rates for class III and IV restorations as 95% and 90%, which corresponded to annual failure rates of around 0.5% and 1%, respectively.[21] A systematic review that investigated the long-term survival of class III and IV anterior restorations showed annual failure rates between 0% and 4.1%.[1] Moura and others reported a lower survival rate (77.8%) than that found in the present study for class IV restorations at three years.[8] However, in the study by Moura and others,[8] the restorations were placed by undergraduate dental students and the main cause of failure was restoration loss due to poor adhesiveness. The authors stated that restoration debonding might have resulted from the operators' lack of experience with the adhesives, coupled with the stresses generated at the incisal angle, which challenge the tooth-restoration interface of class IV restorations.[8]

In the present study, two Filtek Supreme XT restorations failed because of a fracture in the restoration. One Filtek Supreme XT was lost. According to the evaluation criteria, these three restorations received delta scores for marginal adaptation. Likewise, all three Ceram X duo restorations failed because of a fracture in the restoration. These restorations were also rated delta for marginal adaptation. Although four Filtek Supreme XT and three Ceram X duo restorations exhibited marginal discoloration at five years, this discoloration was clinically acceptable (bravo) and did not cause failure in the restorations. In contrast to the current findings, Deliperi reported alpha scores for all class III/IV microhybrid composite restorations regarding

Table 5: *Results of clinical evaluation of class IV composite restorations using modified USPHS criteria*

	Recall Rate	Retention		Color match			Marginal discoloration		
		A	C	A	B	C	A	B	C
Baseline									
FiltekSupreme XT/Scotchbond Multipurpose	100.0 (42)	100.0 (42)	—	100.0 (42)	—	—	100.0 (42)	—	—
Ceram X duo/XP Bond	100.0 (42)	100.0 (42)	—	92.9 (39)	7.1 (3)	—	100.0 (42)	—	—
One year									
FiltekSupreme XT/Scotchbond Multipurpose	94.1 (39)	100.0 (39)	—	100.0 (39)	—		100.0 (39)	—	—
Ceram X duo/XP Bond	94.1 (39)	100.0 (39)	—	92.3 (36)	7.7 (3)		100.0 (39)	—	—
Two years									
FiltekSupreme XT /Scotchbond Multipurpose	82.4 (34)	97.1 (33)	2.9 (1)	100.0 (33)	—	—	93.9 (31)	6.1 (2)	—
Ceram X duo /XP Bond	82.4 (34)	97.1 (33)	2.9 (1)	90.9 (30)	9.1 (3)	—	97.0 (32)	3.0 (1)	—
Three years									
FiltekSupreme XT/Scotchbond Multipurpose	79.4 (32)	93.9 (31)	6.1 (2)	100.0 (31)	—	—	93.5 (29)	6.5 (2)	
Ceram X duo/XP Bond	79.4 (32)	90.9 (30)	9.1 (3)	90.0 (27)	10.0 (3)	—	96.7 (29)	3.3 (1)	
Four years									
FiltekSupreme XT /Scotchbond Multipurpose	70.6 (27)	93.1 (27)	6.9 (2)	92.6 (25)	7.4 (2)	—	85.2 (23)	14.8 (4)	
Ceram X duo /XP Bond	70.6 (26)	89.7 (26)	10.3 (3)	84.6 (22)	15.4 (4)	—	92.3 (24)	7.7 (2)	
Five years									
FiltekSupreme XT /Scotchbond Multipurpose	70.6 (27)	86.2 (25)	13.8 (4)	92.0 (23)	8.0 (2)	—	84.0 (21)	16.0 (4)	
Ceram X duo /XP Bond	70.6 (26)	89.7 (26)	10.3 (3)	84.6 (22)	15.4 (4)		88.5 (23)	11.5 (3)	

Observations are shown as percentages (cumulative number of restorations). A, alpha; B, bravo; C, charlie; D, delta.

marginal discoloration and marginal integrity.[24] Häfer and others reported that at 48 months, 4 of 45 class III/IV restorations, with two-step etch-and-rinse adhesive, failed due to marginal integrity (two restorations), marginal discolorations (one restoration), and loss of vitality (one restoration).[25] In these two previous studies,[24,25] class III and IV restorations were evaluated together. Therefore, cavity location and size, as well as variability of the bonding substrate, may reflect the variation in failure rates reported in the literature. A meta-analysis reported that the failure rate of class IV restorations was twice that of class III restorations at 10 years (10% vs 5%).[21] In a maximum 14-year follow-up study of class IV restorations, fracture was the main cause of failure, which occurred in 11 of 43 (25.6%) composite restorations (Pekafil).[22] Another study reported that esthetics (43%), and anatomic form (26%), were the main reasons that anterior restorations (class III and IV) failed, whereas fracture (22%) had an annual failure rate of between 0.5% and 1.8% during a maximum of 20 years.[2] Thus, although the evaluation period of these two above mentioned studies were longer (14 and 20 years, respectively) [2,22] than our study (five years), fracture was a common reason for failure across all three studies.

It has been stated that loss of material or fracture failures in class IV restorations are probably directly associated with increased wear and incisal stresses.[22] A systematic review on the long-term survival of anterior class III and IV restorations reported that tooth or restoration fracture was the most frequent cause of failure among the studies.[1] Class IV restorations that involve the incisal edge in anterior teeth are subjected to high masticatory loads, and hence, fracture is a possible clinical outcome.[1,2,8] Furthermore, most class IV restorations do not include mechanical retention, which may lead to challenges associated with the bond interface of the restoration.[1] Thus, these aforementioned assumptions were probably the reasons that fracture was the main cause of failure for the Filtek Supreme XT and Ceram X duo restorations.

No restorations in the present study exhibited failure due to marginal discoloration. In support of our findings regarding marginal adaptation and marginal discoloration, it has been reported that marginal integrity is not linked to the method or system of tooth conditioning, which confirms that detectable margins do not necessarily mean there will be stained margins.[21] Also, the bonding to enamel is essential for a good seal and the prevention of marginal discoloration because 100% of the visible margin of class III/IV restorations is usually

Table 5: *Results of clinical evaluation of class IV composite restorations using modified USPHS criteria (ext.)*

	Wear/Anatomic Form			Caries		Marginal adaptation				Surface texture			
	A	B	C	A	B	A	B	C	D	A	B	C	D
Baseline													
FiltekSupreme XT/Scotchbond Multipurpose	100.0 (42)	—	—	100.0 (42)	—	100.0 (42)	—	—	—	100.0 (42)	—	—	—
Ceram X duo/XP Bond	100.0 (42)	—	—	100.0 (42)	—	100.0 (42)	—	—	—	100.0 (42)	—	—	—
One year													
FiltekSupreme XT/Scotchbond Multipurpose	92.3 (36)	7.7 (3)	—	100.0 (39)	-	100.0 (39)	—	—	—	100.0 (39)	—	—	—
Ceram X duo/XP Bond	97.4 (38)	2.6 (1)		100.0 (39)	-	100.0 (39)	—	—	—	100.0 (39)	—	—	—
Two years													
FiltekSupreme XT /Scotchbond Multipurpose	90.9 (30)	9.1 (3)		100.0 (33)	—	100.0 (33)	—	—	—	100.0 (33)	—	—	—
Ceram X duo /XP Bond	97.0 (32)	3.0 (1)		100.0 (33)	—	97.1 (33)	—	—	2.9 (1)	100.0 (33)		—	—
Three years													
FiltekSupreme XT/Scotchbond Multipurpose	90.3 (28)	9.7 (3)		100.0 (31)	—	96.9 (31)	—	—	3.1 (1)	100.0 (31)		—	—
Ceram X duo/XP Bond	96.7 (29)	3.3 (1)		100.0 (30)	—	93.8 (30)	—	—	6.3 (2)	100.0 (30)		—	—
Four years													
FiltekSupreme XT /Scotchbond Multipurpose	88.9 (24)	11.1 (3)		100.0 (27)	-	100.0 (27)	—	—	—	100.0 (27)		—	—
Ceram X duo /XP Bond	96.2 (25)	3.8 (1)		100.0 (26)	-	100.0 (26)	—	—	—	100.0 (26)			
Five years													
FiltekSupreme XT /Scotchbond Multipurpose	88.0 (22)	12.0 (3)		100.0 (25)	—	92.6 (25)	—	—	7.4 (2)	100.0 (25)		—	—
Ceram X duo /XP Bond	96.2 (25)	3.8 (1)		100.0 (26)	—	100.0 (26)	—	—	—	100.0 (26)		—	—

located in the enamel. The use of 37% phosphoric acid in enamel etching remains the most successful approach to form a microretentive pattern, which permits good bonding to ground enamel.[21,26] In the present study, the nanofilled (Filtek Supreme XT) and nanohybrid (Ceram X duo) composites were used in combination with their manufacturer-recommended etch-and-rinse adhesives, which may have contributed to the lack of failed restorations, with regard to marginal discoloration. A meta-analysis regarding the clinical effectiveness of direct anterior restorations concluded that less discoloration was seen at restoration margins when enamel etching was performed with phosphoric acid compared with restorations that used other conditioning systems.[21]

At five years, there was no statistically significant difference between the nanofilled and nanohybrid composite restorations, with regard to the color match. However, the nanofilled composite (Filtek Supreme XT) restorations were considered to have a better color match than the nanohybrid composite restorations. In contrast, our previous study found a comparably greater restoration percentage for a color match at four years, in which the same composites were used to restore space closure in buildup restorations on anterior teeth.[14] This difference may be associated with the difference in restoration type. In our previous study,[14] restorations were applied only to the enamel, whereas the present study included enamel and dentin as the bonding substrates. Conversely, other studies that used a microhybrid or a highly filled hybrid composite material showed more color change than the present study at three or five years,[8,24] which could be due to differences between the teeth and operator skills. In one of these previous studies, the restorations were applied to endodontically treated and bleached teeth,[24] whereas in the other study, the restorations were performed by undergraduate dental students.[8] Another contributing factor may be the differences between size and type of composite fillers used. It has been stated that the initial gloss of many restoratives was quite good, but in hybrid composite (microhybrids, nanohybrids), plucking of the larger fillers, caused loss of gloss.[3] In contrast, the nanoclusters were sheared at a rate similar to the surrounding matrix during abrasion in the nanofilled composite, allowing the restorations to maintain a smoother surface for long-term polish retention,[3] which could explain the improved color match in the nanofilled compared with nanohybrid composite restorations at five years, as found in the current study.

Although no statistically significant difference was found between the nanofilled and nanohybrid composites, the nanofilled composite restorations exhibited a lower percentage of ideal restorations than the nanohybrid composite restorations, with respect to

wear. However, a similar percentage of ideal restorations were previously obtained in buildup restorations for the same composite materials on anterior teeth at four years.[14] Conversely, a higher percentage of ideal restorations was found in class III/IV restorations or buildup restorations on anterior teeth at three, four, and five years.[8,14,24] These conflicting percentages of ideal restorations may be due to differences among the types of composites used and/or restoration types. A previous meta-analysis on the clinical effectiveness of direct anterior restorations reported that the loss of anatomic form was material dependent.[21]

In the present study, neither the nanofilled nor nanohybrid composites exhibited caries adjacent to their margins. These findings are in accordance with previous studies.[8,14,24] A low rate of secondary caries (4 of 43 Pekafil restorations) was observed in class IV restorations in a maximum 14-year follow-up study.[22] Also, secondary caries were largely absent in a maximum 20-year clinical study of anterior restorations that included class III and IV cavities.[2] Differences in evaluation times between these two studies and our study may account for the slight difference in the rates of secondary caries because a longer evaluation time may increase the risk of caries. It was reported that caries adjacent to the restoration were infrequent and seen less often in anterior restorations.[1,21] Therefore, anterior restorations are probably more likely to be replaced because of esthetic demands, trauma fracture, and loss of retention, which may explain the different functional demands of anterior and posterior teeth.[1] Moreover, etch-and-rinse adhesives were used in the current study for the nanofilled and nanohybrid composites. Enamel etching with phosphoric acid reduces the occurrence of marginal discoloration, which, in turn, may reduce the replacement of restorations due to the confusion between stained margins and caries at the margin or may be due to esthetic reasons.[21]

In the present study, all of the nanofilled and nanohybrid composite restorations, showed ideal (alpha-rated) surface texture. These findings are in accordance with previous studies.[14,24] In addition, the dental nanocomposite system showed equivalent translucency, polish, and polish retention properties to those of microfill composites and maintained the physical and wear resistance of several hybrid composites.[23] Therefore, the use of nanotechnology-based modern composites in the present study could have resulted in improved surface texture.

CONCLUSIONS

Despite the limited number of restorations, no statistically significant differences were found between the nanofilled and nanohybrid composite restorations in any of the clinical criteria evaluated. The main cause of restoration failure was restoration fracture. Modern nanofilled and nanohybrid composites may provide good long-term results in class IV cavities. However, more long-term clinical studies are warranted.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ethical Committee of Istanbul University. The approval code for this study is 2008/697.

Conflict of Interest Declaration

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

(Accepted 11 June 2017)

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Double-blind Randomized Study to Evaluate the Safety and Efficacy of Over-the-counter Tooth-whitening Agents Containing 2.9% Hydrogen Peroxide

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Clinical Relevance

This study supports the safety and efficacy of over-the-counter tooth-whitening products containing 2.9% hydrogen peroxide.

ABSTRACT

Objectives: In this double-blind randomized study, we evaluated the safety and efficacy of over-the-counter (OTC) bleaching products that included 2.9% hydrogen peroxide (H_2O_2) with two methods of application: strip and paint-on.

Methods and Materials: A commonly used product was selected for each type (strip and paint-on) of OTC bleaching agent. In total, 75 volunteers were assigned randomly into five groups: two test groups (strip and paint-on), two negative control groups (products without H_2O_2), and one positive control group (dentist-supervised home bleaching). The tooth shade was evaluated with a spectrophotometer and Vita shade guide at baseline and 2 weeks and 4 weeks after use. To document any adverse reactions, such as hypersensitivity or tissue irritation, all patients were examined and the Gingival Index (GI), Plaque Index (PI), and a visual analog scale (VAS) measuring the cold response were obtained.

Results: There were significant differences among the five groups ($p<0.001$). The positive control showed the greatest color changes; then, in decreasing order, the strip-type test group, paint-on-type test group, and negative controls. The strip-type bleaching agent was significantly more effective than the paint-on-type agent and the negative control, while it

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DOI: 10.2341/16-379-C

was significantly less effective than the dentist-supervised home bleaching. Regardless of the treatment group, the canines showed greater color changes than did the central or lateral incisors. Some cases of gingival irritation and hypersensitivity were observed, but they were mild and reversible. GI, PI, and VAS scores were not significantly changed.

Conclusions: Within the limitations of this study, the results indicated that the strip-type and paint-on-type OTC bleaching agents were significantly less efficacious than was dentist-supervised home bleaching; however, they showed acceptable safety and efficacy. The strip-type was more effective than was the paint-on-type in this study.

INTRODUCTION

Because of increased demand for tooth bleaching and interest in dental esthetics, various bleaching products and methods have been introduced. Among such products is a dentist-supervised home bleaching system with a custom tray. The product was developed by Haywood and Heymann and offers an innovative approach to the field of tooth bleaching.[1] Since 2000, affordable and convenient over-the-counter (OTC) products have been manufactured for the comfort of consumers. OTC products are categorized based on the delivery method of the bleaching agent, and include prefabricated trays, whitening strips, and paint-on applications.[2] Products are also available as toothpaste, chewing gum, mouth rinse, and floss[3]; however, those products have lower efficacies compared with strips or paint-on products.[3]

Despite the increasing use of OTC at-home bleaching agents, they may not be as effective as dentist-supervised bleaching because of their low concentrations of hydrogen peroxide (H_2O_2).[2-6] Additionally, incorrect use of these OTC agents could lead to adverse effects.[2-6] In addition, there have been few well-organized clinical trials examining the efficacy and side effects of OTC bleaching products. This is particularly true for products containing less than 3% H_2O_2.[2,4,7-9] Thus, the present study was conducted to evaluate the efficacy and safety of some commonly used OTC bleaching products based on two methods of application (strip and paint-on), comparing them with negative controls that did not contain H_2O_2. The null hypothesis was that there are no differences in the effectiveness of different application methods.

METHODS AND MATERIALS

Commercially available tooth-whitening products were classified according to their application methods. A negative control containing the same ingredients as the selected product, except for H_2O_2, was manufactured, and for a positive control, a dentist-supervised home-bleaching product was used. In total, 75 subjects were recruited who participated in this randomized, double-blind, and placebo-controlled study.

Commercially Available Tooth-Whitening Products

Initially, the tooth-whitening products were categorized into strip, paint-on, and gel-tray products, and a common product was selected from each group. Claren White Now strips (LG Household and Health Care, Seoul, Korea), and White Now dental whitening pen gels (LG Household) were chosen as the strip and paint-on products, respectively. The same manufacturer also provided a test gel-tray product that included 2.9% H_2O_2. However, the gel-tray product was excluded from this study due to adverse effects during a preliminary study. Specifically, when the gel-type bleaching agent directly contacted the oral mucosa, it caused soreness.

The selected strip and paint-on products contained 2.9% H_2O_2. Negative controls containing the same ingredients as the product, except for the whitening agent H_2O_2, were used in the same way as the test groups. As a positive control, a dentist supervised home-bleaching product, the Opalescence tooth whitening gel (Ultradent Products Inc, South Jordan, UT, USA) was used; it contained 10% carbamide peroxide, which is equivalent to 3.62% H_2O_2. Treatments were performed twice daily for 30 minutes each, and continued for 4 weeks according to the manufacturer's directions and the results of previous studies.[8,10]

Selection of Participants

Volunteers over 19 years old interested in tooth bleaching were recruited to participate after obtaining approval from the institutional review board of the Ewha Womans University Hospital. Maxillary and mandibular anterior teeth of volunteers with good systemic and oral health and mild-to-moderate tooth discoloration, screened through oral examinations and questionnaires, were enrolled. Subjects with extensive resin, porcelain restorations, dental caries and wear, or hypersensitivity due to gingivitis or periodontitis were excluded (Table 1).

Table 1: *Criteria Used for Selection and Exclusion of Participants*

Inclusion Criteria	Exclusion Criteria
1. Good systemic and oral health	1. No informed consent
2. Moderate and relatively mild tooth discoloration	2. Insufficient teeth for bleaching
3. Informed consent from adults over 19 years of age	3. Resin or porcelain restoration in the anterior dentition
4. Understands the purpose of the experiment	4. Pulpal inflammation due to dental caries or wear
	5. Hypersensitivity due to gingivitis or periodontitis
	6. Excessive discoloration due to drugs or congenital developmental disorder

The experiment was designed to be carried out with seven groups (three test groups, three negative controls, and one positive control), but due to complications in a preliminary study, the gel tray–type product was removed from the experiment. Thus, there were, in total, 75 participants, 15 in each of the five groups (two test groups, two negative controls, and one positive control). The recruited subjects were randomly assigned to groups through a code provided by SAS software. Of the 75 participants, 64 were female, and the overall average age was 30.3 years (±5.95 years).

Application and Analysis of Tooth-Bleaching Agent

During the first visit, informed consent was obtained after an explanation of the study, and the corresponding tooth-bleaching agent was provided once the subject was assigned randomly to a group. To ensure blinding of the research team, products were sealed in identical opaque containers, and numbered by an independent researcher. Thus, neither the investigators nor the participants knew which treatment was administered. Both verbal and written tooth-bleaching agent application instructions and precautions were given, and the participants were told to call immediately in case of an adverse reaction. In total, three visits, including the first, were scheduled, and tooth shade improvement and adverse reactions were evaluated.

Visit 1 (Screening and Baseline)—After an explanation and discussion of the experiment, the volunteers were screened using the selection and exclusion criteria. Written consent, demographic information, and patient histories were collected from the subjects. Baseline measures were assessed, and the tooth-bleaching agents were provided after random assignment to a group. Each participant received an identifying number as well as a numbered product in a sealed, opaque container. Written instructions were also provided detailing the storage and application protocols. Participants were instructed to avoid food and drinks that might stain teeth, including tea, coffee, red wine, and red fruits. To standardize oral hygiene, participants received soft-bristled, adult toothbrushes and toothpastes that lacked whitening agents. Participants were instructed to brush their teeth three times daily for at least 3 minutes each time for 4 weeks. A researcher not involved in collecting tooth-color measurements administered the tooth-bleaching products to each patient. Tooth-color measurements were taken by one evaluator using a spectrophotometer, and two or three others using a Vita shade guide. The evaluators who measured tooth color did not know to which group each patient belonged.

Visit 2 (2 Weeks)—After using the bleaching agent for 2 weeks, an effectiveness evaluation was performed. To document any adverse reactions, such as hypersensitivity or tissue irritation, all patients were examined through an ocular inspection, detailed questions, hypersensitivity test, and photographic record.

Visit 3 (4 Weeks)—After using the bleaching agent for 4 weeks, an effectiveness evaluation was performed, and adverse reactions were documented in the same way as for visit 2.

Evaluation of Clinical Efficacy

A spectrophotometer, accepted as the gold standard for verifying the effectiveness of bleaching agents, and a Vita shade guide, the most commonly used shade guide in the clinic, were used to measure changes in tooth shade. All examinations were performed at the same location using the same light source while participants assumed the same position.

CIE Lab* Spectrophotometer Measurements

After calibration of the spectrophotometer (SpectroShade Micro, Verona, Italy), the head of the instrument was placed near and perpendicular to the middle third of the labial surface of the anterior tooth to assess its shade. CIE Lab* was selected for color specification mode, and measurements were taken three times each and averaged to be used for that tooth's CIE Lab* value. To calculate the similar

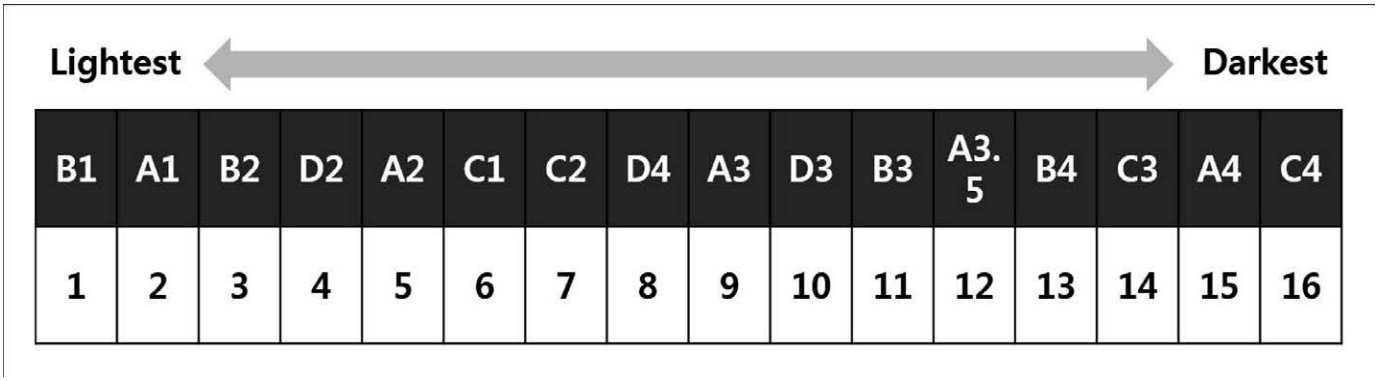

Figure 1. *Numeric scores of Vitapan Classical shade guide in decreasing order of brightness.*

ΔE, the measured L*, a*, b* values were entered in the equation below.

$$\Delta E^* = ((L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2)^{1/2}$$

where ΔE is the shade difference; L_1, a_1, and b_1 are the CIE Lab* values before bleaching; and L_2, a_2, and b_2 are the CIE Lab* values after bleaching. The lightness (L) value represents brightness, ranging from 0 to 100, and the number directly increases with the brightness. A* and b* values indicate chroma (+a* [red], −a* [green], +b* [orange], −b* (blue]), ranging from −60 to +80, and the larger the absolute value, the more saturated the color.

Vita shade

According to the traditional method of selecting the shade most closely matching the tooth, hue (designated as A, B, C, D) was chosen followed by the chroma (designated as numbers 1 through 4). The tooth shade can be classified into 16 steps, and each step was given a numerical score, which was used to calculate the difference in the numbers before and after bleaching (Figure 1). The shades were chosen using the same method for the 12 maxillary and mandibular anterior teeth. Two independent researchers determined the shade at the same time and location. When there was a disagreement, a more experienced third examiner participated in the decision. Prior to examination, all investigators were calibrated using the Vita shade guides (Vitapan Classical shade guide, Vita Zahnfabrik, Bad Säckingen, Germany) and using the sample of 10 patients with characteristics similar to those of the study subjects. All investigators correctly assigned shades in at least 85% of cases.

Safety Assessment

During the first visit, the Gingival Index (GI), Plaque Index (PI), and a visual analog scale (VAS) measuring the cold response were obtained, and these values continued to be recorded in the follow-up visits to evaluate the safety of the tooth whitening agents.

Gingival Index (GI) by Löe and Silness—Score Criteria: 0, no inflammation; 1, mild gingival inflammation and change in color, slight edema, no bleeding on probing; 2, moderate inflammation and glazing, erythema, bleeding on probing; 3, severe inflammation, erythema and hypertrophy, ulceration, spontaneous bleeding tendency.

Plaque Index (PI) by Löe and Silness—Score Criteria: 0, absence of plaque; 1, film of plaque present on the free gingival margin and adjacent area of the tooth. Plaque observed only after using disclosing solution or a probe on the tooth surface; 2, moderate accumulation of plaque in the gingival pocket, margin, and surrounding tooth surface, observed with the naked eye; 3, abundance of plaque in the gingival pocket and margin.

Visual Analog Scale (VAS)—The intensity of discomfort felt by the patient was marked on a 100-mm horizontal line with the numbers 0 (no pain) and 10 (severe pain) at each end. All subjects marked a vertical line indicating the severity of hypersensitivity to cold, tested with an ice stick.

Statistical Analysis

Statistical analyses were performed using the SPSS software (ver. 21.0; SPSS, Inc, Chicago, IL, USA). Tooth color changes were analyzed using a linear mixed model, regardless of tooth location. Statistical analysis of color change for each tooth type was

Table 2: *Spectrophotometric Analysis After Bleaching for 2 Weeks*

Tooth Location		Group					
		DSHB[a]	Strip Type (Test)	Strip Type (-)[b]	Paint-On Type (Test)	Paint-On Type (-)	*P* Value
Upper	Central incisor	3.20 ± 1.32^{Ac}	2.61 ± 1.16^{AB}	0.93 ± 0.41^{C}	1.58 ± 0.85^{BD}	1.32 ± 0.98^{CD}	<0.001
	Lateral incisor	4.19 ± 1.96	2.82 ± 1.09^{A}	1.36 ± 0.58^{B}	1.59 ± 0.97^{AC}	1.21 ± 0.98^{BC}	<0.001
	Canine	6.21 ± 2.51	4.58 ± 1.66	1.70 ± 0.80^{A}	2.26 ± 1.42^{B}	1.50 ± 0.71^{AB}	<0.001
Lower	Central incisor	3.07 ± 1.47	1.87 ± 0.87^{AB}	1.13 ± 0.93^{AC}	1.64 ± 0.98^{BD}	0.99 ± 0.62^{CD}	<0.001
	Lateral incisor	3.92 ± 1.73	2.35 ± 1.02^{A}	0.95 ± 0.40^{B}	1.66 ± 1.04^{AC}	1.14 ± 0.51^{BC}	<0.001
	Canine	5.44 ± 2.35	3.47 ± 1.46	1.57 ± 0.77 ^{A}	1.88 ± 0.83^{B}	1.46 ± 0.71^{AB}	<0.001
Total		4.34 ± 1.71	2.95 ± 1.02	1.26 ± 0.46^{A}	1.77 ± 0.74^{B}	1.27 ± 1.54^{AB}	<0.001

[a] *DSHB indicates dentist-supervised home bleaching.*
[b] *(-) indicates negative control.*
[c] *Same letters indicate that the values are statistically similar for each row* ($p<0.05$).

analyzed using one-way analysis of variance with Tukey's multiple comparison test. All values were considered statistically significant when $p<0.05$.

RESULTS

Assessment of Shade Improvement

The baseline color parameters for each treatment group were not significantly different. After bleaching, spectrophotometric assessments revealed an increase in L and a decrease in chroma (a and b). Thus, decreased scores were observed using the Vita shade guide. The positive control showed the highest color changes in all tooth positions; then, in decreasing order, the strip-type test group, paint-on-type test group, and negative controls. The strip-type bleaching agent was significantly more effective than was the paint-on-type agent, while it was significantly less effective than the dentist-supervised home bleaching. Regardless of the treatment group, the canine groups showed greater color changes than did the central and lateral incisor groups in both upper and lower jaws (Tables 3-6).

Spectrophotometer

2 Weeks (Table 2 and Figure 2)—In all tooth locations, the positive control showed a significant difference vs the other treatment groups, except for the upper central incisor treated with the strip-type bleaching agent ($p=0.485$). When the strip-type and the paint-on-type were compared, only the canine groups were significantly different (upper: $p=0.01$, lower: $p=0.023$). When the test group was compared with the negative control, there was a significant difference except for the lower central incisor ($p=0.285$) in the strip-type. In contrast, the paint-on-type did not show a significant difference between the test and negative control groups ($p=0.456$).

4 Weeks (Table 3 and Figure 3)—In all tooth locations, the positive control showed a significant difference compared with the other treatment groups, except for the upper central incisor treated with the strip-type bleaching agent ($p=0.466$). Between the strip-type and paint-on-type, unlike the results at 2 weeks, there were significant differences except for the lower incisors (lower central incisor: $p=0.692$, lower lateral incisor:

Table 3: *Spectrophotometric Analysis After Bleaching for 4 Weeks*

Tooth Location		Group					
		DSHB[a]	Strip Type (Test)	Strip Type (-)[b]	Paint-On Type (Test)	Paint-On Type (-)	*P* Value
Upper	Central incisor	4.27 ± 1.36^{Ac}	3.55 ± 1.52^{A}	0.88 ± 0.25^{B}	1.95 ± 1.4^{C}	1.20 ± 0.22^{BC}	<0.001
	Lateral incisor	5.58 ± 1.97	4.11 ± 1.68	1.46 ± 0.79^{A}	1.94 ± 0.95^{B}	1.10 ± 0.79^{AB}	<0.001
	Canine	08.1 ± 2.77	6.16 ± 2.42	2.03 ± 0.79^{A}	2.53 ± 1.48^{B}	1.34 ± 0.81^{AB}	<0.001
Lower	Central incisor	4.02 ± 1.71	2.48 ± 0.29^{A}	1.07 ± 0.90^{B}	1.87 ± 1.51^{AC}	1.38 ± 0.23^{BC}	<0.001
	Lateral incisor	5.01 ± 1.90	3.30 ± 1.38^{A}	1.11 ± 0.47^{B}	2.19 ± 1.41^{AC}	1.44 ± 1.07^{BC}	<0.001
	Canine	7.11 ± 2.87	4.70 ± 2.13	1.70 ± 0.90^{A}	2.26 ± 1.13^{B}	1.50 ± 0.75^{AB}	<0.001
Total		5.68 ± 1.91	4.05 ± 1.48	1.36 ± 0.43^{A}	2.12 ± 1.09^{B}	1.32 ± 0.57^{AB}	<0.001

[a] *DSHB indicates dentist-supervised home bleaching.*
[b] *(-) indicates negative control.*
[c] *Same letters indicate that the values are statistically similar for each row* ($p<0.05$).

Table 4: *Color Changes Based on the Vita Shade Guide After Bleaching for 2 Weeks*

Tooth Location		Group					
		DSHB[a]	Strip Type (Test)	Strip Type (−)[b]	Paint-on Type (Test)	Paint-on Type (-)	*P* Value
Upper	Central incisor	−3.13 ± 1.51^{ABc}	−1.93 ± 1.83^{ACD}	−0.70 ± 1.62^{CDE}	−2.00 ± 1.04^{BD}	−0.23 ± 0.92^{E}	<0.001
	Lateral incisor	−3.43 ± 1.52^{AB}	−2.27 ± 1.83^{AC}	−0.50 ± 2.35^{D}	−2.11 ± 1.64^{BCE}	−0.50 ± 0.85^{DE}	<0.001
	Canine	−4.20 ± 1.95	−2.50 ± 1.72^{A}	−0.80 ± 1.98^{B}	−1.32 ± 1.41^{AC}	−0.17 ± 0.84^{BC}	<0.001
Lower	Central incisor	−2.97 ± 2.07	−0.53 ± 0.74^{AB}	−0.80 ± 1.86^{AC}	−0.42 ± 0.83^{BD}	0.00 ± 1.02^{CD}	<0.001
	Lateral incisor	−2.77 ± 2.17^{AB}	−1.80 ± 1.58^{ACD}	−0.73 ± 1.79^{CE}	−1.00 ± 1.63^{BDF}	−0.63 ± 1.20^{EF}	0.005
	Canine	−3.20 ± 2.11^{A}	−2.00 ± 2.15^{AB}	0.37 ± 2.23^{C}	−0.96 ± 1.60^{BD}	0.33 ± 1.17^{CD}	<0.001
Total		−3.28 ± 1.35^{A}	−1.84 ± 1.01^{AB}	−0.52 ± 1.39^{C}	−1.30 ± 0.87^{BD}	−0.25 ± 0.43^{CD}	<0.001

[a] *DSHB indicates dentist-supervised home bleaching.*
[b] *(-) indicates negative control.*
[c] *Same letters indicate that the values are statistically similar for each row* ($p<0.05$).

$p=0.173$). The strip-type bleaching agent was significantly brighter than the negative control at all tooth locations (lower central incisor: $p=0.027$, others: $p<0.001$), but the paint-on-type was not significantly different from the negative control for any tooth location using the bleaching agent for 2 weeks more ($p=0.408$).

Vita shade guide

2 Weeks (Table 4 and Figure 4)—The positive control was better than the strip-type only on the upper canines and lower central incisors (upper canine: $p=0.045$, lower central incisor: $p<0.001$). Also, compared with the paint-on-type, the positive control was more effective on the upper canines and lower central incisors and canines (upper canines and lower central incisors: $p<0.001$, lower canine: $p=0.019$). The strip-type and the paint-on-type were not significantly different ($p>0.309$). The strip-type was significantly different from the negative control on the upper lateral incisors and canines and the lower canines (upper lateral incisor: $p=0.046$, upper canine: $p=0.045$, lower canine: $p=0.009$). The paint-on-type was not significantly different, compared with the negative control, except for the upper central incisor ($p=0.012$).

4 Weeks (Table 5 and Figure 5)—For the upper central incisor, the positive control, strip-type, and paint-on-types were not significantly different (strip: $p=0.577$, paint-on: $p=0.147$). For lower lateral incisors and canines, the results after 2 weeks of using the bleaching agents showed that the positive control was more effective than the other two types, but after 4 weeks, the positive control and strip-type were not significantly different (lower lateral incisor: $p=0.144$, canine: $p=0.117$). The strip-type and the paint-on-type agents were not significantly different ($p>0.339$). The strip-type agent was significantly effective vs the negative control, but not for the lower incisors (central incisor: $p=0.997$, lateral incisor: $p=0.061$). The paint-on-type agent after 2 weeks was significantly effective only on the upper central incisors. However, after 4 weeks, this agent was significantly effective on all the upper anterior teeth, but not on the lower teeth (lower: $p>0.333$).

Table 5: *Color Changes Based on the Vita Shade Guide After Bleaching for 4 Weeks*

		Group					
		DSHB[a]	Strip Type (Test)	Strip Type (-)[b]	Paint-on Type (Test)	Paint-on Type (-)	*P* Value
Upper	Central incisor	−3.83 ± 1.89^{ABc}	−3.00 ± 1.85^{AC}	−0.53 ± 1.30^{D}	2.50 ± 1.61^{BC}	-0.63 ± 0.77^{D}	<0.001
	Lateral incisor	−5.63 ± 2.07	−2.53 ± 1.60^{A}	−0.50 ± 2.64^{B}	−3.25 ± 2.00^{A}	-1.00 ± 1.24^{B}	<0.001
	Canine	−7.13 ± 2.09	−4.57 ± 2.15^{A}	−1.03 ± 2.41^{B}	−3.14 ± 1.76^{A}	-4.00 ± 1.69^{B}	<0.001
Lower	Central incisor	−3.13 ± 2.13	−0.93 ± 1.22^{AB}	−0.73 ± 1.98^{AC}	−0.75 ± 1.28^{BD}	0.07 ± 1.28^{CD}	<0.001
	Lateral incisor	−3.70 ± 1.82 ^{A}	−2.27 ± 1.59^{ABC}	−0.60 ± 1.59^{BD}	−1.61 ± 1.91^{CE}	-0.73 ± 1.44^{DE}	<0.001
	Canine	−5.57 ± 2.71 ^{A}	−3.67 ± 2.14^{AB}	0.10 ± 1.93^{C}	−1.46 ± 2.34^{BD}	0.03 ± 1.29^{CD}	<0.001
Total		−4.83 ± 1.30	−2.83 ± 1.15^{A}	−0.54 ± 1.40^{B}	−2.12 ± 0.94^{A}	−0.44 ± 0.75^{B}	<0.001

[a] *DSHB indicates dentist-supervised home bleaching.*
[b] *(-) indicates negative control.*
[c] *Same letters indicate that the values are statistically similar for each row* ($p<0.05$).

Table 6: *Changes in VAS Test Values After Bleaching for 2 or 4 Weeks**

Change in VAS	Group				
	DSHB[a]	Strip Type (Test)	Strip Type (-)[b]	Paint-on Type (Test)	Paint-on Type (-)
2 wk	0.23 ± 6.34	−1.45 ± 15.81	3.79 ± 8.70	−1.18 ± 6.78	1.68 ± 5.79
4 wk	−3.43 ± 8.04	−1.03 ± 13.89	−0.52 ± 5.92	−4.09 ± 9.04	−0.39 ± 6.47

[a] *DSHB indicates dentist-supervised home bleaching.*
[b] *(-) indicates negative control.*
* *No statistically significant difference was found between groups or before and after tooth bleaching* ($p>0.05$).

Safety Assessment

Each patient in this study completed the Gingival Index (GI), Plaque Index (PI), and visual analog scale (VAS). All of them scored 0 or 1 on the GI and PI at the first visit. After using the bleaching agents, GI scores of all patients were unchanged, and PI scores on two patients changed. However, although these scores changed, they were not considered indicative of unfavorable conditions because the maximum score was 1, while most other scores were 0. No significant difference was observed in the VAS between groups or before or after bleaching ($p>0.05$; Table 6). Although no severe gingival irritation or hypersensitivity was reported, some mild and reversible cases of oral mucosal irritation and hypersensitivity were observed. Four patients using the paint-on-type test product and four patients using paint-on-type negative control product complained of mucosal irritation, especially on the lower lip mucosa. The symptoms subsided within a few minutes after rinsing out the agent.

DISCUSSION

Although the Vita shade guide is the most commonly used method of measuring changes in tooth color, visually discerning the shade is impacted by various factors, including the light source, color of the gingiva, makeup, angle of measurement, skill of the examiner, and eye fatigue.[11,12] Also, because the Vita shade guide has a limited range of colors, meaning that it cannot provide true CIE Lab* values and changes in shade cannot be accurately measured.[12,13] However, the spectrophotometer is considered the gold standard for evaluating the effectiveness of a tooth bleaching agent, due to its high reproducibility and objectivity.[14] Despite this, reflections on the tooth surface, diameter and direction of the spectrophotometer tip, and the background surrounding the tooth can affect shade matching,[15] so evaluators must be fully aware of spectrophotometer use. Thus, the error range should be minimized through appropriate use of the Vita shade guide and the spectrophotometer, accurate interpretation of results, and repeated measurements. In this study, both methods were used to evaluate changes in shade after bleaching.

These changes were examined using the ΔE value, which was calculated with the coordinates of L, a, and b. A change is considered to be imperceptible to the naked eye when the ΔE value is between 0 and 2, barely perceptible when between 2 and 3, generally perceptible between 3 and 8, and readily perceptible when the value is over 8.[16] Also, it has been suggested that a value below 3.3 is clinically

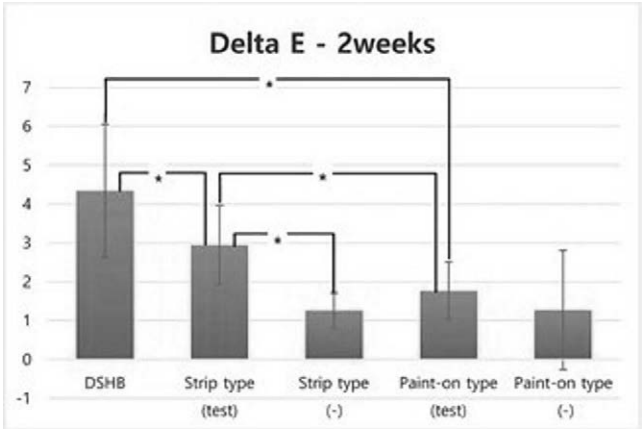

Figure 2. *Spectrophotometer: color changes after bleaching for 2 weeks.* *$p<0.05$.

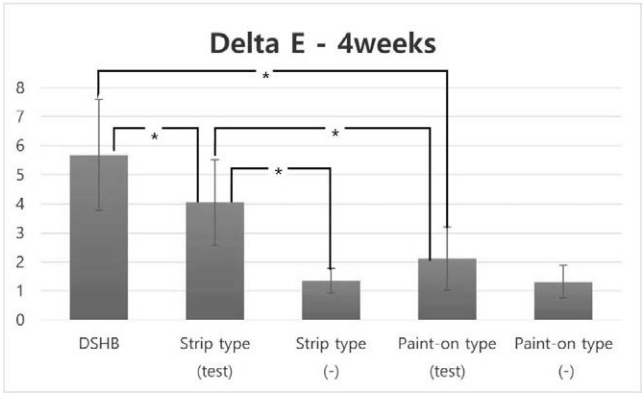

Figure 3. *Spectrophotometer: color changes after bleaching for 4 weeks.* *$p<0.05$.

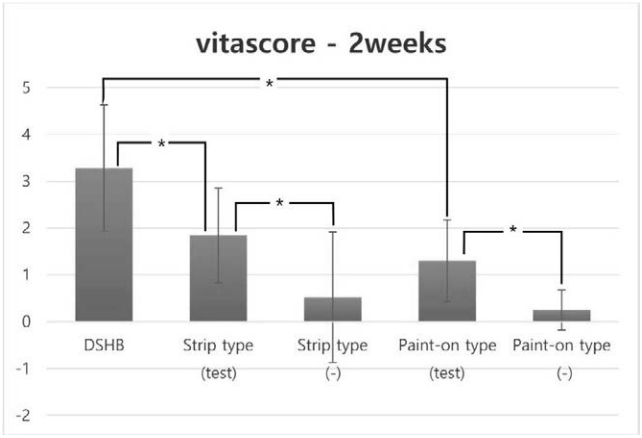

Figure 4. *Vita shade guide: color changes after bleaching for 2 weeks.* *p<0.05.

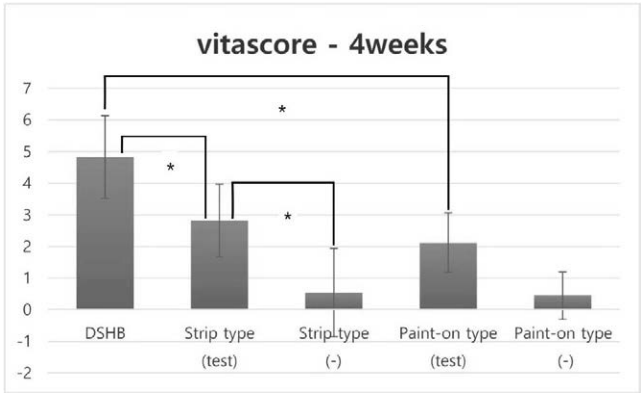

Figure 5. *Vita shade guide: color changes after bleaching for 4 weeks.* *p<0.05.

insignificant, because a change in shade is recognized only when ΔE is >3.3.[17] The International Organization of Standardization (ISO) strongly recommends that external tooth-bleaching products show a ΔE of at least 2.[18] In this study, the strip-type and paint-on-type test groups did show considerable tooth color changes. After 4 weeks of bleaching, the color changes were over 2 in all tooth positions, and the mean value was 4.05 in the strip-type group. In the paint-on group, color changes were around 2, and the mean value was 2.12. These results indicate that the strip-type and paint-on-type OTC tooth-bleaching agents caused perceptible color changes, and the values satisfied the recommendations of the ISO.

In this study, there were larger changes in the canines with darker shades. The amount of shade change, from most to least, was in the order of canine, lateral incisor, and central incisor. However, because canines are usually darker than incisors, it is appropriate to consider that there is greater amount of shade change when the tooth has a darker shade, consistent with the findings of previous studies.[8,19,20] A significant correlation was reported between the magnitude of tooth color changes and b* (yellow-blue) values.[20] Thus, teeth of increasingly dark shades of yellow exhibited greater color changes after bleaching.[19]

According to the present study, improvement in shade change was in the order of positive control, strip, and paint-on. After using the bleaching agent for 4 weeks, all test groups showed ΔE* >2 overall, indicating significant shade improvement. The strip-type agent was significantly more effective than was the paint-on-type, thus, the null hypothesis was rejected. The strip-type agent showed significant improvement vs the negative control; however, the paint-on-type did not. The color change (ΔE) of the paint-on-type was >2, satisfying the recommendations of the ISO. Similar trends were obtained with the Vita shade guide, although it did not show differences between groups in some tooth positions. Within the limitations of this study, the spectrophotometer appeared superior to the Vita shade guide in detecting differences among groups. Nonetheless, visual assessment is still commonly used because of its convenience and low cost.

The two test products showed inferior results vs the positive control, which could be due to differences in the bleaching agent content (positive control: 10% carbamide peroxide, equivalent to 3.62% H_2O_2[12]; strip-type and paint-on-type: 2.9% H_2O_2); however, consistency and application method could have also affected the results.[21,22] The positive control can be evenly applied on the tooth surface because the bleaching agent has a gel-like consistency. Also, the customized tray minimizes unnecessary contact of the agent with the gingiva and protects the agent from saliva and lip movement. The strip-type agent is designed so that the bleaching agent can be protected from saliva and lip movement. However, it is difficult to apply evenly on the tooth surface, especially because the marginal area of the strip is straight. According to the experimental subjects, the mandibular strip fell off readily from the tooth surface. This could have resulted from saliva affecting retention of the strip and making it difficult for the tooth to be exposed sufficiently to the bleaching agent. Also, a minimal change in shade may have resulted from the smaller amount of the bleaching agent in the strip-type product. The absolute quantity of bleaching agent on the strip was limited. The paint-on-type bleaching agent had a gel-like consistency similar to the positive control, but it was not designed to be

protected from the oral environment as was the positive control and the strip-type product.

Although it is supposed to produce a coating layer when dried, the bleaching agent is thought to be readily removed when in contact with the oral mucosa and to lead to gingival irritation.[7,8] All patients who complained of gingival irritation were in the paint-on-type agent groups. The irritation may not be caused by the H_2O_2, because the same number of patients in both the test and negative control paint-on groups complained of reversible and mild mucosal irritation. Although the paint-on-type had the same H_2O_2 content as the strip-type, it resulted in an inferior shade improvement due to the application method. According to the results, the negative controls also resulted in improvements in tooth shade. This indicates that agents other than H_2O_2 have the potential to change shades.[23] Thus, when investigating the efficacy of a bleaching agent, one should test the product without H_2O_2 rather than use a negative control without any of the components contained in commercially available products to confirm the effect of H_2O_2 concentrations.

Major factors affecting the efficacy of tooth bleaching are concentration of whitening agents and application time.[12,24-27] The degree of color change was smaller in this study, compared with those observed previously. Such differences were due to the lower concentrations of H_2O_2 (2.9%) used in this study, compared with previous studies (6%-10%).[28,29] Higher concentrations of bleaching agents can achieve faster effects but, at least theoretically, when the application time of a product with a lower concentration of bleaching agent is extended, its efficacy can be similar to the highly concentrated bleaching agent. However, practically, it will be difficult to achieve an efficacy similar to a highly concentrated product with a low-concentration product because the relationship between the concentration of bleaching agent and number of applications needed to achieve optimal efficacy have an exponential regression relationship rather than a linear one.[27] In this study, as the use of bleaching agent was repeated, tooth colors were improved but the degree of change decreased.

CONCLUSIONS

- Shade improvement (ΔE) was more than 2 in all test groups, and was in the order of positive control, strip-type, and paint-on-type agent.
- The null hypothesis was rejected because the strip-type was significantly more effective than the paint-on-type.
- However, both OTC tooth-bleaching agents showed significantly lower efficacy than did the dentist-supervised home bleaching kit.
- The tested bleaching agents did not affect PI or GI and caused only minimal and reversible mucosal irritation and hypersensitivity, showing relative safety.

Acknowledgement

This research was supported by a grant (14172MFDS307) from the Ministry of Food and Drug Safety in 2014.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ewha Womans University Hospital. The approval code for this study is 14-10A-14.

Conflict of Interest

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

(Accepted 24 August 2017)

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Laboratory Research

Effect of Resin Luting Systems and Alumina Particle Air Abrasion on Bond Strength to Zirconia

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Clinical Relevance

Alumina particle air abrasion (topographical alterations) of the inner surface of zirconia-based ceramic (Y-TZP) restorations and the application of universal primers (adhesives) containing multiple bond promoters (methacrylate monomers, including phosphate monomers such as 10-methacryloyloxi-decyl-dihydrogen-phosphate and silane) optimize the adhesion of Y-TZP to resin cements.

SUMMARY

This study aimed to evaluate the effect of different primer/resin luting agent combinations and alumina air abrasion on the adhesion to zirconia. Eighty blocks (4×4×3 mm) of Lava Frame Zirconia (3M ESPE) were produced and randomly assigned into eight groups (n=10) according to two zirconia surface treatments (untreated or air abrasion with 50-μm alumina particles) and four luting systems (SU: Scotchbond Universal/RelyX Unicem 2; ZP: Z-Prime Plus/Duo-link Universal; MB: Monobond Plus/Variolink II; and AP: Alloy Primer/ED Primer II/Panavia F 2.0). After the conditioning and primer applications, resin luting agents were manipulated and applied on the zirconia, using a matrix, to form a cylinder (2 mm in diameter×2 mm high), followed by photoactivation for 40 seconds. After that, the specimens were stored in distilled water (37 °C) for 120 days and then submitted to shear bond strength testing, followed by failure mode evaluation under an optical microscope (30×). A two-way analysis of variance and Tukey test (α=0.05) were used for data analysis. Alumina air abrasion (Al) promoted higher bond values for the three luting systems, except for SU, which showed the best results without air abrasion, while with air abrasion, Al-SU, Al-ZP, and Al-MB presented higher values compared to Al-AP. We concluded that the alumina air abrasion of zirconia

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DOI: 10.2341/15-352-L

surfaces seemed to be dispensable for the SU group, while air abrasion (topographical alterations) enhanced the adhesion of the ZP, MB, and AP groups.

INTRODUCTION

The use of zirconia-based ceramics (Y-TZP) has increased over the past decade, especially due to their superior mechanical and esthetic properties used as frameworks and monolithic restorations in posterior areas.[1-5] Despite the large clinical application of zirconia ceramics, the major drawback is related to its unpredictable bond with resin cements.[1-3,6] Zirconia is a densely sintered ceramic that offers chemical and dimensional stability and desirable physical properties, such as high flexural strength, high modulus of elasticity, and high fracture toughness compared to other ceramic materials.[4] On the other hand, polycrystalline ceramics are nonetchable by hydrofluoric acid since it does not contain amorphous silica in its composition.[4-6] Therefore, traditional surface treatment methods indicated for silica-based ceramic, such as hydrofluoric acid followed by silane coupling agent application, are impracticable on zirconia-based ceramics due to their high crystalline content and silica-free surfaces.[2,4-6]

The use of conventional cements, such as zinc phosphate or resin-modified glass ionomer cements, had been initially recommended by the manufacturers for luting zirconia restorations.[4] However, adhesive cementation has been shown to increase fracture resistance[4] and the fatigue resistance[7] and improve the longevity of ceramic restorations[8] besides sealing internal surface defects created by airborne particle abrasion.[9] Achieving a reliable adhesion to zirconia-based ceramics would further expand the application of this material.[4] However, it requires surface treatments based on physical and/or chemical treatments, such as air abrasion and zirconium oxide dedicated primers, that will promote the interaction between zirconia and luting substrate.

Air abrasion with aluminum oxide particles aims to roughen the internal surface of the ceramic restorations to optimize the adhesion area and promote better mechanical interlocking with the resin cement.[4,5] Although studies have shown that particle air abrasion promotes the improvement of resin bond to zirconia materials,[10,11] previous studies reported that this treatment creates surface microcracks and defects that can damage the mechanical properties of the material.[12-15] Hence, the use of zirconia primers has been studied as a substitute to air abrasion in order to promote the chemical bond to zirconia through phosphate monomers without a mechanical bond.

Phosphate monomers act as bifunctional molecules in which one end connects with the ceramic's metal oxides (such as aluminum and zirconium), while the other end copolymerizes with the resin cement matrix. Some examples of these bifunctional monomers are 10-methacryloyloxydecyl-dihydrogenphosphate (10-MDP), 2-methacryloyloxyethyl dihydrogen, and 6-methacryloyloxyhexyl dihydrogen phosphate .[16] It has been found that MDP monomers promote a water-resistant chemical bond to densely sintered zirconia ceramics.[5,17] MDP monomers are also available in some resin cements, such as RelyX Unicem (3M ESPE, Maplewood, MN, USA) and Panavia (Kuraray Noritake Dental Inc, Chiyoda-ku, Tokyo, Japan). Today, several primers that claim chemical adhesion to zirconia are available on the market. However, more studies are necessary to verify their efficacy and long-term bond durability when combining different primers and resin cements.[18]

Thus, the aim of this study was to evaluate the effect of alumina particle air abrasion and different luting systems (primer/resin luting agent) on bond strength to zirconia after aging. The research hypotheses were 1) that regardless of the luting system adopted, alumina air abrasion would promote bond improvement and 2) that there would be no difference in bond strength among the different luting systems.

METHODS AND MATERIALS

The resin cements and primers used in this study are shown in Table 1. The evaluator was "blind" for some study conditions (cementation procedure, shear test, failure analysis).

Specimen Preparation

Eighty blocks (4×4×3 mm) Lava Frame (3M ESPE) zirconia were obtained, sintered as recommended by manufacturer, and embedded in autopolymerizing acrylic resin cylinders (Orthodontic Resin, Dentsply Caulk, Milford, DE, USA), keeping free a zirconia surface for bonding procedures. After the resin acrylic polymerization, the zirconia's exposed surface was ground finished with 800-grit silicon carbide abrasive (Auto Advanced, 3M ESPE) under running water in a polishing machine (Buehler Metaserv, Buehler, Düsseldorf, Germany) for 1 minute. After-

Table 1: *Information and Chemical Composition of Resin Cements and Zirconia Primers/Bonding Agents Used in the Present Study*

Commercial Brand	Lot Number	Specifications
RelyX Unicem2 Translucent, 3M ESPE	524950	Base paste: methacrylate monomers containing phosphoric acid groups, initiator components, silanated fillers, stabilizers, rheological additives. Catalyst paste: methacrylate monomers, alkaline (basic) fillers, silanated fillers, initiator components, stabilizers, rheological additives, pigments
Scotchbond Universal, 3M ESPE	525058	MDP phosphate monomer, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane.
Duo-link Universal, Bisco	1400003516	Base paste: Bis-GMA, triethyleneglycol dimethacrylate, Glass Filler. Catalyst past: Bis-GMA, triethyleneglycol dimethacrylate, glass filler
Z-Prime Plus, Bisco	1400002857	Biphenyl dimethacrylate, MDP, ethanol
Variolink II Transparent, Ivoclar Vivadent	Base: T00900; catalyst: T00901	Base: 26.3%wt monomer (Bis-GMA, urethane dimethacrylate, triethylene glycol dimethacrylate), 73.4%wt filler. Catalyst: 22.0%wt monomer, 77.2%wt filler
Monobond Plus, Ivoclar Vivadent	S55075	Alcohol solution of silane methacrylate, phosphoric acid methacrylate, sulfide methacrylate
Panavia F2.0 Light, Kuraray	061229	Paste A: 10-methacryloyloxydecyl dihydrogen phosphate, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic methacrylate, hydrophilic aliphatic dimethacrylate, silanated silica filler, silanated colloidal silica, dl-camphorquinone, catalysts, initiators, others Paste B: sodium fluoride, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic methacrylate, hydrophilic aliphatic dimethacrylate, silanated barium glass filler, catalysts, accelerators, pigments, others
Alloy Primer ED Primer II, Kuraray	Alloy Primer: 0436AA ED Primer II Liquid A: 00322B Liquid B: 00196A	Alloy primer: acetone, VBATDT,[a] 10-MDP ED Primer II Liquid A: 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate, N-methacryloyl-5-aminosalicylic acid, water, accelerators ED Primer II Liquid B: N-methacryloyl-5-aminosalicylic acid, water, catalysts, accelerators

[a] *Phosphate monomer 6-(4-vinylbenzyl-N-propyl)amino-1,3,5-triazine-2,4-dithione.*

ward, the specimens were ultrasonically cleaned for five minutes in deionized water and then wiped with 95%vol ethanol.

Zirconia specimens were randomly allocated into eight groups (n=10), as described in Table 2. Half of these groups did not receive zirconia surface treatment, while the other groups had the zirconia surface air abraded (aluminum oxide, 50 microns per 15 second, device sample distance of 10 mm, pressure of 87 psi, perpendicular to the surface, by linear motion), using a microetcher (Optiblast, Buffalo DentalManufacturing Inc, New York, NY, USA).

Cementation Procedure

After particle air abrasion, the primer agents were applied over the treated zirconia surface following the manufacturer's instructions (Table 2). A cylinder of resin cement was built on the ceramic surface using an Ultradent SBS device (Bisco, Schaumburg, IL, USA) with an inner diameter of 2 mm and height of 2 mm. The resin cements were manipulated following the manufacturer's instructions (Table 2). Resin cement cylinders were light cured (Bluephase style, Ivoclar Vivadent, Schaan, Liechtenstein) with an intensity of 1330 mW/cm^2 for 40 seconds. All specimens were prepared by the same operator to avoid interoperator variability.

Shear Bond Strength Test

After four months of storage in distilled water at 37°C for aging process, all specimens were submitted to the shear bond strength test in a universal test machine (Compact force gauge, Bisco) at a crosshead speed of 0.5 mm/min, using a flat rod as testing assembly (Figure 1). The bond strength R (MPa) was calculated using the following formula: $R = F/A$, where F is the load for specimen failure (N) and A is the cross-sectional interfacial area (mm^2).

Failure-Type Analysis

After the shear bond testing, the debonded surfaces were observed through an optical microscope (Microview Canada, Markham, ON, Canada) at 30× magnification to determine and classify the failure mode. The failure types were classified into the following categories: (A) adhesive at the zirconia-cement interface and (B) cohesive in the resin cement structure. Micrographs of representative

Table 2: *Testing Groups According to the Zirconia Surface Treatment and Cementation Strategy. Bonding Procedures, as Recommended by Manufacturers, Are Described as Footnotes*

Cementation Strategy	Zirconia Surface Treatment	
	Without	With
Scotchbond Universal/RelyX Unicem 2[a]	SU	Al-SU
Z-Prime Plus/Duo-link Universal[b]	ZP	Al-ZP
Monobond Plus/Variolink II[c]	MB	Al-MB
Alloy Primer/ED Primer II/Panavia F 2.0[d]	AP	Al-AP

[a] *Scotchbond Universal was applied on zirconia surface, with a microbrush, for 20 seconds and then air-dried for 5 seconds. The specimen was positioned on the Ultradent SBS device, and RelyX Unicem cement was manipulated by equal parts of both pastes.*
[b] *Two layers of Z-Prime Plus were applied over the zirconia surface and air-dried for 5 seconds. Then the specimen was positioned on the Ultradent SBS device, and the Duo-link Universal cement was manipulated by equal parts of both pastes.*
[c] *Initially, Monobond Plus was applied on the specimen-free surface and let stay for 60 seconds, then it was air-dried to remove possible primer excess. The specimen was positioned on the Ultradent SBS device, and the Variolink II cement was manipulated by equal parts of both pastes during 10 seconds.*
[d] *First, Alloy Primer was applied with a microbrush and let dry by itself. Then ED Primer II (A and B) was applied due its influence on resin cement polymerization, let stay for 30 seconds, and then gently air-dried. Finally, the specimen was positioned on the Ultradent SBS device, and Panavia cement was manipulated by equal parts of both pastes mixed for 20 seconds.*

samples were taken using a scanning electron microscope (S-2500 Hitachi Scanning Electron Microscope, Hitachi High Technologies America, Inc, Schaumburg, IL, USA).

Data Analysis

Statistical analysis was performed using the software Statistix 8.0 for Windows (Analytical Software Inc, Tallahassee, FL, USA). Bond strength data (MPa) were subjected to two-way analysis of variance (ANOVA) and Tukey tests (α=0.05); p-values less than 0.05 were considered to be statistically significant in all tests. Multiple comparisons were made by repeated measures tests at a significance level of 0.05.

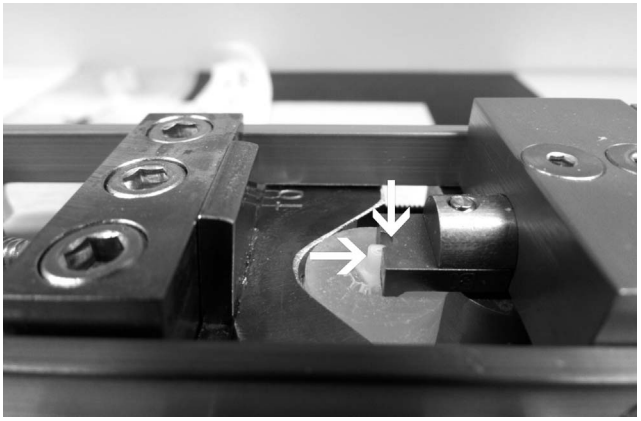

Figure 1. *Picture of the test setup adopted for the shear bond strength testing. It notes the proper fit between the resin cement cylinder (→), built over the zirconia block, and the flat rod testing assembly (⇓).*

RESULTS

Two-way ANOVA revealed that the cementation approach ($p<0.0001$) and the zirconia surface treatment ($p<0.0001$; Al_2O_3>untreated) showed a significant effect on the bond strength between the zirconia and the resin cement. Interaction between the factors surface treatment and cementation strategy ($p=0.0062$) was also significant.

Surface treatment by particle air abrasion (Al_2O_3) statistically increased bond strength values for all luting systems, except for Scotchbond Universal/RelyX Unicem 2 (Table 3).

Comparing the different luting systems without air abrasion, Scotchbond Universal/RelyX Unicem 2 promoted the highest bond values, while Z-Prime Plus/Duo-link Universal and Monobond Plus/Variolink II were intermediaries, and Alloy Primer/ED Primer II/Panavia F 2.0 showed the lowest bond strength values (Table 3). When surface treatment with aluminum oxide particles was performed, it potentiated the bonding of Z-Prime Plus/Duo-link Universal and Monobond Plus/Variolink II cementation strategies, which achieved values statistically similar to Scotchbond Universal/RelyX Unicem 2 but higher than Alloy Primer/ED Primer II/Panavia F 2.0 (Table 3).

No pretest failures occurred in this study. The percentages of adhesive and cohesive failure after test are represented in Table 3. Representative micrographs of tested samples are shown in the Figure 2.

DISCUSSION

The first hypothesis (bond improvement with the use of alumina air abrasion regardless of the luting system) was partially accepted once the zirconia surface treatment increased bond strength values when compared to untreated surface, except for the luting system that used Scotchbond Universal/RelyX Unicem 2.

Air abrasion with aluminum oxide particles with a cross section of 50-125 mm is considered as the main zirconia surface treatment prior to the cementation procedure due to the results achieved and ease of chair-side procedures.[19-21] Alumina air abrasion aims to roughen the zirconia surface, producing microretentions and increasing the adhesion ar-

Table 3: *Data Analysis of Shear Bond Strength Test Results. Presented are the means and Standard Deviations (MPa) and Tukey Tests of Testing Groups. Percentages of Adhesive (A) and Cohesive (B) Failures in Each Group Are Also Described*[a]

Cementation Strategy	Bond Results		Failure Types	
	Alumina Air Abrasion			
	Without	With	Without	With
Scotchbond Universal/RelyX Unicem 2	19.6 ± 5.6 Aa	23.2 ± 5.2 Aa	A: 60%	A: 40%
			B: 40%	B: 60%
Z-Prime Plus/Duo-link Universal	11.1 ± 6.3 Bb	21.2 ± 5.2 Aa	A: 40%	A: 30%
			B: 60%	B: 70%
Monobond Plus/Variolink II	11.8 ± 3.3 Bb	25 ± 7.1 Aa	A: 70%	A: 40%
			B: 30%	B: 60%
Alloy Primer/ED Primer II/Panavia F 2.0	4.7 ± 1.3 Bc	9.1 ± 2 Ab	A: 100%	A: 100%
			B: 0%	B: 0%

[a] *Different uppercase letters mean statistical difference between surface treatment groups, keeping unaltered the cementation strategy; different lowercase letters mean statistical difference among cementation strategy groups, keeping unaltered the surface treatment.*

ea.[4,5,20] In this way, like hydrofluoric acid treatment on silica-based ceramics, alumina air abrasion allows a mechanical interlocking between these acid-resistant ceramics and the resin luting/ceramic primer.

Although air abrasion has been proven to be a successful treatment, enhancing the bond strength,[10,11,22-25] its positive influence on resin adhesion to zirconia is contradictory. Murthy and others[26] evaluated the effect of different surface treatments on shear bond strength between zirconia and resin luting agents and observed no significant differences on bond strength values between control (no surface treatment) and alumina air abrasion (with either 110 μm or 250 μm) groups. Foxton and others[27] obtained a durable (six-month) bond to Procera All Ceram and Procera All Zirkon using a ceramic primer containing MDP-phosphate monomer without any additional surface treatment (alumina air abrasion or erbium laser treated). These findings are in accordance with the findings observed in the present study when considering the Scotchbond Universal/RelyX Unicem 2 luting system, in which air abrasion did not improve the adhesion to zirconia. Air abrasion had a positive role by increasing bonding values for the luting systems with Z-Prime Plus/Duo-link Universal, Monobond Plus/Variolink II, and Alloy Primer/ED Primer II/Panavia F2.0, which is in agreement with several studies.[10,11,22,23,25,28,29] Furthermore, Amaral and others[24] and Inokoshi and others[30] reported that air abrasion is required when luting zirconia, even when using novel primers and universal adhesives. Therefore, the fact that Scotchbond Universal/RelyX Unicem 2 did not present increased bonding values after alumina air abrasion may be related to its chemical composition. This primer has been proposed as a substitute to air abrasion (morphological modifications) for zirconia surface treatment prior to the luting procedure due to the chemical adhesion to zirconia given by its differentiated chemical composition (Table 1), including multiple bond promoters, as reported below.

In this sense, the second null hypothesis (no difference in bond strength among the different luting procedures) was denied since different luting systems resulted in different bond strengths. Without surface treatment with air abrasion, Scotchbond Universal/RelyX Unicem 2 obtained the highest bond strength values. However, when alumina air abrasion was performed, it potentiated the adhesion of Z-Prime Plus/Duo-link Universal and Monobond Plus/Variolink II, resulting in similar bond strength of Scotchbond Universal/RelyX Unicem 2 (groups with the highest bond strength values). Even with an increase in bond strength after air abrasion, Alloy Primer/ED Primer II/Panavia F 2.0 presented the lowest bond values. Recent investigations have reported that the selection of the luting agent is one of the most important factors for luting zirconia restorations.[20,25,31-34]

As mentioned, another alternative surface treatment to zirconia is the use of ceramic primers for the chemical bond between the zirconia surface and the resin luting agent through phosphate monomers. They act as bifunctional molecules and improve the wettability of the ceramic surface and bond strength to resin cements by chemical interaction.[35] Thus, a luting approach that includes phosphate monomers could enhance, for example, bond strength and restoration longevity.[24,25,28,29,32,36-38] Inokoshi and

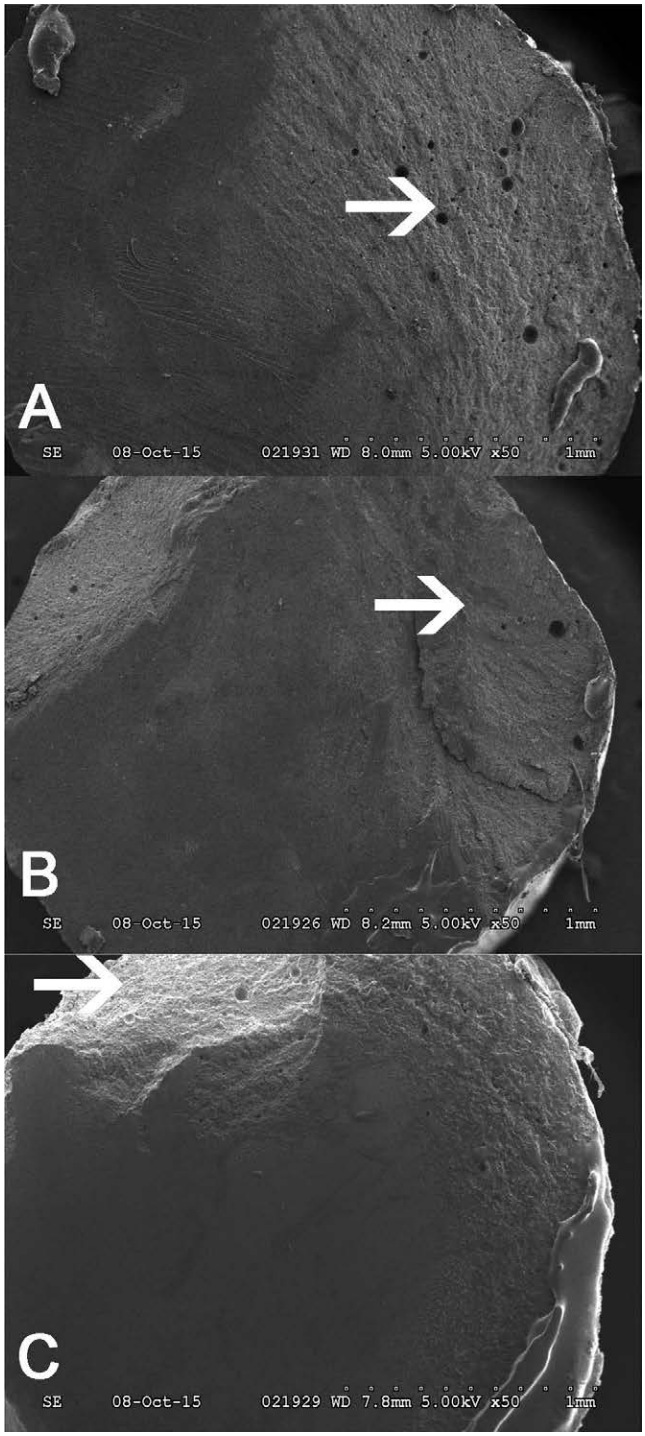

Figure 2. *Representative scanning electron micrographs of the resin cement debonded zirconia surfaces. It notes that the part of resin cement fractured (→) due to typical biomechanical behavior of the shear testing. Similar failure profiles were even observed between groups with the highest (A is representative of the SU group, and B is representative of the SU-Al group) and lowest (C is representative of the AP group) bond strength values.*

others[30] and De Souza and others[35] observed that the application of an MDP-based adhesive might improve the bond strength to zirconia. On the other hand, Cristoforides and others[39] observed that an MDP-containing liner is not effective for zirconia Y-TZP composite repairs. 10-MDP is one of the well-known phosphate monomers. Being in use for more than 20 years, it has achieved promising results in adhesion. Its phosphoric acid group bonds chemically to zirconia atoms, while the double bonds on the other end of the molecule copolymerize with the resin monomers; in addition, they are able to create ionic bonds with calcium from hydroxyapatite.[17,39,40] In the present study, it was observed that primers/adhesives that associate multiple adhesion promoters—10-MDP and others—appear to promote efficient adhesion to zirconia.

Scotchbond, as a universal adhesive, can be used in both tooth and indirect restoration surfaces, including metals, composites, glass-containing ceramics, and nonglass ceramics. This property is given by its composition (Table 1), which contains multiple adhesion promoters, such as methacrylate monomers, 10-MDP, polyalkenoic acid copolymer, and silane. The resin cement RelyX Unicem 2 also presents (Table 1) methacrylate monomers containing phosphoric acid groups. In the current study, this luting approach yielded the highest bonding values, as observed by Amaral and others,[24] De Souza and others,[35] and Seabra and others.[41] This finding was assigned to the adhesive chemical composition, whose MDP molecules may have interacted chemically to zirconium and aluminum (Al_2O_3) oxide particles due to their affinity to metallic oxides. Amaral and others[24] have also observed that Scotchbond Universal was effective in promoting durable bond to zirconia even without previous air abrasion with silica or alumina.

Hence, the present luting system may be a safe alternative for a stable bond to zirconia without the need of additional surface treatments, as the use of air abrasion may generate damage to the zirconia surface, including the presence of microcracks.[12-15] Moreover, as a universal adhesive, it simplifies the clinical procedures, reducing the number of steps involved in an adhesive luting system.[24] The superiority of the luting system using Scotchbond Universal, despite the fact that Panavia F 2.0 (Table 1), Alloy Primer, and ED Primer II (Table 1) also present 10-MDP and other phosphate monomers, methacrylates, and dimethacrylates in their compositions as adhesion promoters, was also observed previously.[25,42] On the other hand, several authors

have observed that the Panavia luting system was superior to other luting agent/primer associations, such as AZ Primer/ResiCem (Shofu, Kyoto, Japan),[43] Metal/Zirconia Primer/Multilink (Ivoclar Vivadent),[43] Monobond S/Multilink (Ivoclar Vivadent),[34] and Porcelain Liner M/SuperBond (SunMedical Co, Moriyama, Japan).[34] Furthermore, Piwowarczyk and others[44] reported superior adhesion of RelyX Unicem and Panavia F2.0 to zirconia after aging when compared to zinc phosphate and modified glass-ionomer cements.

The inferior results achieved by Panavia F in the present study might have been affected by bonding procedures. During the bonding protocol, the Alloy Primer was applied first on the zirconia surface, followed by ED Primer. The manufacturer recommends ED Primer to be applied over the remaining tooth structure to keep contact with both tooth structure and the Panavia luting agent and recommends Alloy Primer to be applied over the zirconia to react with its surface and with the Panavia luting agent. Although no teeth have been used in the present study, as mentioned, ED Primer was applied after Alloy Primer and may have interfered with the reaction between Alloy Primer and Panavia. A study by Özcan and others[34] had also observed inferior or no adhesion to zirconia after artificial aging when following the manufacturer's instructions regarding Panavia F2.0. During initial tests for the present study, it was attempted to remove ED Primer II from the Alloy Primer/Panavia F2.0 luting protocol, but no adhesion to zirconia was achieved.

The occurrence of cohesive failures by shear testing can be considered as a limitation of this investigation since it may lead to misinterpretation of the bond performance of tested materials.[45-47] Instead of an indication of strong bonding, cohesive failures are explained by the mechanics of the test and the brittleness of the materials involved.[45] It may affect the accurate assessment of the interfacial bond strength, precluding a correct evaluation of each studied variable effect. Another limitation was the absence of thermocycling since only water storage at 37°C was performed, even though 150 and 300 days of distillated water storage might be a useful method for aging cement-zirconia adhesion interfaces, producing similar results to water storage associated with 12,000 thermocycling cicles.[48]

Although the use of ceramic primers seems to be part of a promising luting protocol by enabling to reduce time-consuming and critical clinical steps, more studies are still necessary to confirm the long-term efficiency of primers as bond promoters to zirconia.

CONCLUSION

The topographical alterations of the zirconia surface via alumina particle air abrasion provided enhanced resin bonding to the zirconia surface. The use of universal primers (adhesives) containing multiple bond promoters (methacrylate monomers, phosphate monomers such as 10-MDP, and silane), such as Scotchbond Universal, is a promising alternative to improve the adhesion of resin luting agents to zirconia.

Acknowledgements

CAPES/MITACS and PIBIC/CNPq agencies financially supported the present study.

Conflict of Interest

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

(Accepted 14 April 2016)

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Bond Stability of a Universal Adhesive System to Eroded/ Abraded Dentin After Deproteinization

MG Augusto • CRG Torres • CR Pucci • N Schlueter • AB Borges

Clinical Relevance

Sodium hypochlorite deproteinization may be a viable technique to enhance bond strength and maintain stability to eroded and eroded/abraded dentin.

SUMMARY

Objective: Erosive/abrasive challenges can potentially compromise bonding to dentin. Aiming to improve the quality and stability of bonding to this substrate, this study investigated the combined effect of erosion and toothbrush abrasion on the microtensile bond strength (μTBS) stability to dentin using a universal adhesive system in total and self-etching modes, associated or not associated with deproteinization.

Methods: Bovine dentin specimens were divided into five groups according to the organic matrix condition (n=20): control (C); erosion (E); erosion + abrasion (EA); erosion + sodium hypochlorite (EH); erosion + abrasion + sodium hypochlorite (EAH). The groups were further divided (n=10) according to the mode of application (total or self-etching) of a universal adhesive. After the bonding procedure, composite blocks were built up, and the samples were cut to obtain sticks for μTBS testing. For each specimen, one-half of the sticks was immediately tested, and the other one-half was tested after artificial aging (5000 thermocycles, 5°C and 55°C).

Results: Three-way analysis of variance (α=5%) showed a significant difference for the triple

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DOI: 10.2341/16-173-L

interaction (p=0.0007). Higher μTBS means were obtained for the EH and EAH groups compared with the E and EA groups. The control group showed immediate μTBS values similar to that of the E and EA groups for both bond strategies.

Conclusions: Erosion and erosion/abrasion did not significantly influence the immediate μTBS to dentin. Artificial aging reduced μTBS values for the groups C, E, and EA using the total-etching mode. Deproteinization maintained the bond stability to artificially aged eroded and eroded/abraded dentin.

INTRODUCTION

In the last decades, the emergence of therapies based on the preventive properties of fluorides allowed a significant decline in the prevalence of caries and, consequently, an increase in tooth longevity.[1,2] However, the growing consumption of acidic beverages leads to an increase of erosive wear risk, especially in adolescents.[3,4]

Recurrent erosive challenges can result in the loss of enamel and exposure of dentin. In the case of dentin erosive wear, restorative procedures may be necessary to recover esthetics and/or function, protect the remaining tooth structure, and prevent hypersensitivity.[5] Since erosive lesions are normally shallow and flat surface defects,[6] retention of the restorations will be mainly determined by the bond strategies used.

The surface of eroded dentin lacks minerals and presents a mesh of collagen fibrils (organic matrix).[7] Studies have shown that this organic matrix is able to slow down erosion progression, meaning that, to some extent, it has a protective effect against further erosive episodes.[8] Interestingly, the organic matrix is notably resistant to toothbrushing abrasion.[9] Toothbrushing with forces up to 4 N is not able to remove the organic matrix, but as previously observed,[10] this collagen mesh undergoes compression as the intensity of the force increases.

The compression reduces the interfibular spaces between the collagen fibrils, thereby increasing the density of the organic matrix, which may act as a physical barrier for adhesive penetration. Over time, the collagen fibers that were not impregnated by the adhesive will be susceptible to hydrolysis, creating areas rich in water at the hybrid layer, which favors nonuniform stress distribution and the development of interfacial defects.[11]

The recently developed universal adhesive systems can be used with or without previous acid etching. Most of them contain acidic functional monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), that bind directly to calcium and phosphate ions from the dental hard tissues, forming heavily bound nanolayers.[12,13] This stronger chemical interaction may be able to improve the bonding stability to eroded dentin.

One possibility to cope with the problem of the barrier represented by the thick eroded organic matrix is to deproteinize the eroded dentin surface, allowing a direct interaction of the adhesive to the intact dentin. The classic deproteinization technique proposes the application of sodium hypochlorite after acid etching in order to remove exposed collagen fibrils, producing a mineral surface similar to enamel.[14] Therefore, the removal of the organic matrix of an eroded dentin surface would potentially improve the quality of the adhesive interface as well as the bonding durability.[15-17] Nevertheless, there is a lack of evidence supporting this hypothesis.

Thus, this study investigated the combined effect of erosion and toothbrush abrasion on the immediate and post-aging microtensile bond strength (μTBS) to dentin, using a universal adhesive system in total and self-etching modes, associated or not associated with deproteinization.

The null hypothesis tested was that dentin deproteinization with sodium hypochlorite does not affect immediate or post-aging μTBS to eroded or eroded/abraded dentin when a universal adhesive system is applied with either bonding strategy (total or self-etching mode).

METHODS AND MATERIALS

Sample-Size Calculation

A pilot study was conducted to obtain mean and standard deviation data used to calculate the effect size (f). With the parameters of 0.05 as the significance level and 0.8 as the power test, using the G Power 3.1 statistical analysis software (Heinrich-Heine-Universität, Düsseldorf, Germany); a total of nine specimens per group was determined necessary. Taking into account the potential losses during the experiment, 10 specimens per group were prepared.

Specimen Preparation

One hundred recently extracted bovine incisors stored in 0.1% thymol solution, pH 7.0, at 4°C,

were used. The roots were removed using a diamond disc coupled to a low-speed handpiece, and the crowns were embedded in self-curing acrylic resin (Jet Classico, São Paulo, Brazil) inside polyvinyl chloride rings. The labial surfaces were ground flat using silicon carbide sandpaper P120 grit (Extec Corp, Enfield, CT, USA) coupled to a circular polishing machine (Panambra, São Paulo, Brazil) under constant water cooling, until an area of approximately 6 mm^2 of dentin was exposed. In order to standardize the surface roughness and smear layer, the specimens were polished in the circular polishing machine at a speed of 600 rpm for one minute using silicon carbide sandpaper P600 grit (Extec Corp) under constant water cooling, with a load of 6N (ISO/TS 11405, 2015).

Experimental Group Division

The specimens were randomly assigned into five groups according to the organic matrix condition (n=20): control (C); erosion (E); erosion + abrasion (EA); erosion + sodium hypochlorite (EH); erosion + abrasion + sodium hypochlorite (EAH). The specimens of the control group remained in artificial saliva, four hours a day, for five days. During the intervals of the procedures, all specimens remained stored in 100% relative humidity.

Erosion

The eroded groups were exposed to an erosive cycling procedure consisting of immersion in 0.5% citric acid (natural pH 2.6) for five minutes, four times a day, for five consecutive days. After each immersion, the specimens were rinsed with deionized water for 20 seconds and stored in artificial saliva for 1 hour. Artificial saliva was composed of 0.002 g/L ascorbic acid; 0.030 g/ L glucose; 0.580 g/L NaCl; 0.225 g/L $CaCl_2 \cdot H_2O$; 0.160 g/L NH_4Cl; 1.270 g/L KCl; 0.160 g/L NaSCN; 0.330 g/L KH_2PO_4; 0.200 g/L urea; and 0.426 g/L $Na2HPO_4 \cdot 2H_2O$.[18]

Abrasion

The abrasion was performed twice daily (once after the first erosive impact and again after the last one), 15 seconds per specimen, using an automatic toothbrushing machine (SEM-2T, Odeme Dental Research, Luzerna, Brazil) that performed 30 strokes at a frequency of 2 Hz. A slurry containing fluoride-free toothpaste (Tom's of Maine, Kennebunk, ME, USA) and artificial saliva at a ratio of 1:3 (w/w) was used.[10] A brushing force of 2 N was applied.[19] Soft toothbrushes (Sanifill Ultra Profissional, Hypermarcas, São Paulo, Brazil) with nylon bristles and rounded tips were used. The brushes were fixed in the machine so that the long axes of the toothbrushes were at an angle of 12° to the direction of brushing, avoiding the formation of tracks induced by the bristles on the dentin surface.[20]

Bonding Procedures

The groups were further divided into subgroups (n=10) according to the mode of application of the adhesive system: total-etching or self-etching. The universal adhesive system FuturaBond M+ (Voco, Cuxhaven, Germany) was applied in all groups. For the total-etching mode, a 37% phosphoric acid gel (Condac 37, FGM, Joinville, Brazil) was applied for 15 seconds and rinsed for 15 seconds; the dentin surface was then blot-dried using absorbent paper. For the self-etching mode, no additional etching was used.

When associated with the self-etching mode, the deproteinization was performed before the adhesive application. When associated with the total-etching mode, the deproteinization was performed after the acid etching. The 10% w/v sodium hypochlorite solution (pH 12) was actively applied and rubbed with a disposable applicator (Microbrush, KG Sorensen, Barueri, Brazil) for 60 seconds. The specimens were rinsed for 15 seconds and blot-dried using absorbent paper.[21]

The adhesive was actively applied for 20 seconds, following the manufacturer's instructions, air-dried for 10 seconds, and light-cured using a light-emitting diode device (Radii-cal, SDI, Bayswater, Australia) with an irradiance of 800 mW/cm^2 for 10 seconds.[17] Figure 1 shows the distributions in the experimental groups.

Blocks of composite (GrandioSO, Voco) were built up in two increments on the specimens' surfaces using a silicone matrix (6×6×4 mm). Each increment was light-cured for 40 seconds. After removal of the matrix, additional light-curing was performed for 40 seconds in two opposed lateral sides of the blocks. The specimens were stored in deionized water for 24 hours at 37°C to allow the postcuring of the composite. Table 1 shows the specifications of the materials used for the restorative procedures.

Artificial Aging

In order to obtain sticks with a sectional area of approximately 1 × 1 mm, specimens were mounted

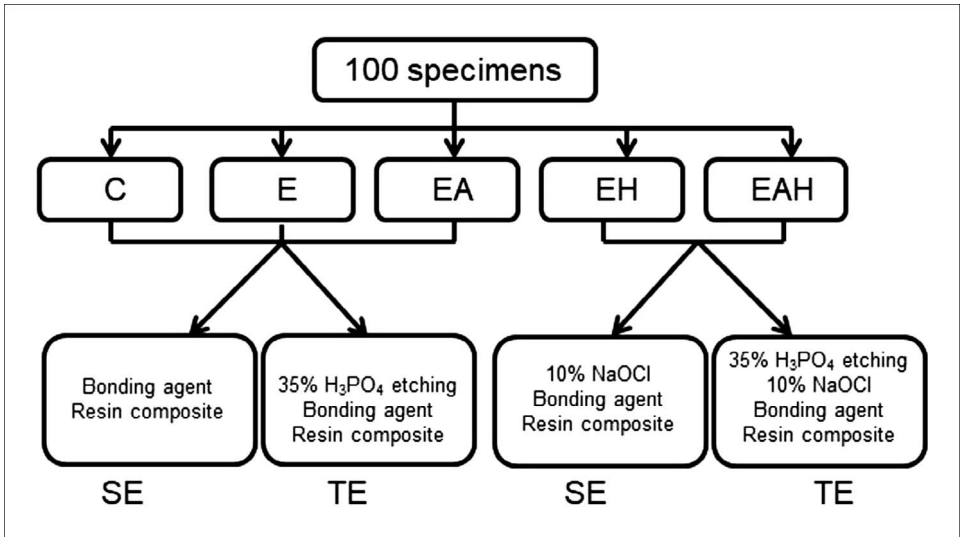

Figure 1. *Division of the samples according to each subgroup (n=10 each). C: control; E: erosion, EA: erosion + abrasion; EH: erosion + sodium hypochlorite; EAH: erosion + abrasion + sodium hypochlorite.*

in a serial cutting machine (Labcut 1010, Extec Corp) and sectioned using a water-cooled diamond disc.

One-half of the sticks obtained from each specimen were submitted to thermocycling (5000 cycles, 5°C-55°C, with the dwell time set at 15 seconds) before the microtensile test in order to simulate approximately 6 months of aging.[22] If an odd number of sticks was obtained from one specimen, the supernumerary stick was forwarded to the artificial aging one-half.

Microtensile Bond Strength Test

The areas of the adhesive interfaces were measured with a digital caliper (accuracy: 0.001 mm; Mitutoyo, Kawazaki, Japan) and the values were used to calculate the bond strength values in MPa. The sticks were individually fixed on metallic holders using cyanoacrylate gel (Loctite 454, Henkel, Düsseldorf, Germany). The microtensile test was performed in a universal testing machine (DL-200MF, EMIC, São José dos Pinhais, Brazil), with a 10 kg load cell, at a constant speed of 1 mm/min.

Failure Mode

Failures were classified as adhesive, cohesive in dentin, cohesive in resin, or mixed through optical stereomicroscopic observation with 60× magnification (Discovery V20, Karl Zeiss, Jena, Germany). Specimens that debonded during preparation or artificial aging (premature failures) were considered for the failure mode analysis but not for the mean μTBS values.

Statistical Analysis

The statistical unit was considered the mean bond strength value obtained from the sticks of each tooth. Thus, statistical analysis was based on the mean bond strength of all the teeth from each subgroup. The results indicated that the residuals were normally distributed and, by plotting against pre-

Table 1: *Materials Used in Restorative Procedures*

Product	Composition	Manufacturer	Batch Number
Condac 37	Phosphoric acid gel 37%	FGM, Joinville, Brazil	131213
Futurabond M+	UDMA, HEMA, MDP, CQ, BHT, and ethanol.	Voco, Cuxhaven, Germany	503138
GrandioSO	Resin matrix: Bis-GMA, BisEMA, TEGDMA, CQ, Amina, BHT.	Voco, Cuxhaven, Germany	427541
	Inorganic content: Nanoparticles of SiO_2: 20-40 nm; Glass ceramic: 1 μm		
	Filler content: 89% (w/w) and 73% (v/v)		
10% sodium hypochlorite	10% Sodium hypochlorite	Quimesp Química Ltda, Guarulhos, Brazil	59146

Abbreviations: BHT, butylated hydroxytoluene; BisEMA, ethoxylated bisphenol-A dimethacrylate; Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; CQ, camphorquinone; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogenphosphate, TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

dicted values, the uniformity was checked. None of the analysis of variance (ANOVA) assumptions were violated. Thus, three-way ANOVA was applied to evaluate the effects of the organic matrix condition, bond strategy, and artificial aging on μTBS, as well as their interaction. Post hoc pairwise comparisons were performed using the Tukey test. GraphPad Software (version 6.01, 2012) was used for the calculations, with a significance level of 5%.

Scanning Electronic Microscopy

In order to provide a qualitative analysis of the dentin surfaces, additional specimens of each subgroup were prepared for scanning electron microscopy evaluation. Crowns of two bovine incisors were used, and five dentin specimens were obtained from each crown (one for each tested group). Their labial surfaces were polished with P600 silicon carbide sandpaper (Extec Corp) and treated as previously described. On the specimens of one crown, the adhesive system was applied according to the total-etching mode, and on the specimens of the other crown the self-etching mode was used. No light-curing was performed. For complete removal of the adhesive, the specimens were placed in plastic tubes and immersed in 2 mL of acetone for 10 minutes in an ultrasonic cube, with one change of the acetone after 5 minutes. The specimens were kept in a desiccation chamber for one week to remove any remaining water. Then, they were placed on aluminum stubs, sputter coated with gold (Emitech SC7620 Sputter Coater, Moorestown, NJ, USA), and observed in a scanning electron microscope (Inspect S50, FEI, Hillsboro, OR, USA) operating at 15-20 KV.

RESULTS

Microtensile Bond Strength

Three-way ANOVA showed significant bond strength differences for the organic matrix condition (p=0.0001), artificial aging (p=0.0001), and the interaction between them (p=0.0007). It did not show a significant difference for the bond strategy (p=0.7843).

The mean μTBS data (MPa, standard deviations in parenthesis) of the tested groups and results of the Tukey test for the triple interaction are shown in Table 2.

It is possible to observe that erosion associated or not associated with toothbrush abrasion, although presenting overall lower numerical μTBS means, did not statistically differ from that of the control group. Deproteinization with 10% sodium hypochlorite increased the bond strength of these groups.

Before aging, the bond strength means of each subgroup were similar for the total-etching and self-etching modes. Artificial aging decreased the bond strength values of groups C, E, and EA using the total-etching mode but not after deproteinization (EH and EAH).

Failure Analysis

In groups C, E, and EA, the failure mode was predominantly adhesive, followed by mixed and cohesive in dentin. In groups EH and EAH, the failure mode was predominantly cohesive in dentin, followed by adhesive and mixed. After artificial aging, an increased number of premature failures was observed (Figures 2 and 3), especially in groups E and EA.

Scanning Electron Microscopy

When the universal adhesive was applied using the self-etching mode, it is noticeable that the dentinal tubules are occluded by a smear layer in the control group (Figure 4A) and opened in the other subgroups (Figures 4B-E). When using the total-etching mode, the dentinal tubules are opened in all subgroups (Figures 4F through J). Regardless of the adhesive mode used, the deproteinized groups presented a characteristic surface pattern of a funnel shape around the dentinal tubules.

DISCUSSION

The main finding of this study was that deproteinization can improve bond strength and maintain the bond stability to eroded and eroded/abraded dentin, thus rejecting the null hypothesis.

Physical and chemical mechanisms are associated with the improved bond strength values observed in groups EH and EAH. The original deproteinization technique (acid etching + sodium hypochlorite) increased the bond strength values[23,24] due to the ability of sodium hypochlorite to remove the collagen network exposed by the acid etching and the organic content from the mineralized underlying dentin. This exposes an extensive labyrinth of secondary tubules and anastomoses that can be penetrated by the adhesive,[16,25] increasing the substrate area of interaction and, consequently, improving the micromechanical retention of the adhesive.[17,26]

The tubule entrances of etched and deproteinized dentin have a characteristic funnel shape. This occurs because the peritubular dentin presents a

Table 2: *Mean Microtensile Bond Strength Data (Standard Deviation) and Results of the Tukey Test for All Subgroups*[a]

	C	E	EA	EH	EAH
Immediate					
SE	21.87 (5.20)AB ab	19.45 (4.80)AB b	18.17 (3.66)A b	29.98 (2.88)A a	30.29 (6.59)A a
TE	25.74 (7.03)A ab	26.42 (6.53)A ab	20.12 (5.83)A b	29.65 (5.79)A a	29.28 (5.05)A a
After aging					
SE	15.99 (3.15)B bc	13.10 (4.04)BC c	12.98 (4.49)AB c	23.62 (2.91)A ab	24.56 (5.62)A a
TE	13.95 (5.56)B b	7.10(5.03)C b	6.87(3.97)B b	25.85 (6.43)A a	25.28 (5.14)A a

Abbreviations: C, control; E, Erosion; EA, erosion + abrasion; EAH, erosion + abrasion + sodium hypochlorite; EH, erosion + sodium hypochlorite; SE, self-etching; TE, total-etching.
[a] *Different uppercase letters indicate differences between adhesive applications and aging conditions (in columns). Different lowercase letters indicate differences between organic matrix conditions (in rows) ($p<0.05$).*

higher mineralization than the intertubular dentin.[27] When acid etching is performed, the peritubular dentin is completely dissolved; the intertubular dentin, near the tubule's entrance, is also demineralized. When sodium hypochlorite is applied, it dissolves the exposed collagen around the tubules, creating the funnel shape.[26] In Figure 4 it is possible to see the funnel shape in both the EH and EAH groups. Although phosphoric acid was not applied in the self-etching mode, the dentin surface was previously etched during erosive challenges, creating a similar aspect.

The dentin deproteinization exposes numerous hydroxyapatite crystals that can potentially react with the adhesive monomer. The tested universal adhesive contains the functional monomer 10-MDP, which has a high affinity to deproteinized dentin and forms 10-MDP-Ca salts.[13] This chemical interaction may be one of the factors responsible for providing bond stability when using the universal adhesive in both modes.

Limited information is available regarding bonding to eroded dentin, and controversial results have been found. Previous studies observed lower bond strength values to eroded dentin than to sound dentin.[28-31] However this finding was not observed in the results of the present study, which is in agreement with the findings of other studies.[32,33] This divergence may be related to the differences in the aggressiveness of the erosive challenge (exposure time, pH of acid solution, type, and concentration of acid), storage time in the remineralizing solution, composition of the remineralizing solution, and adhesive system used. Moreover, the presence of the organic matrix depends on the moment that the last erosive challenge occurred. If it occurred shortly before the adhesive application, the collagen matrix will be present, but over time, the collagen fibrils can be partially or completely degraded by dentin-derived proteolytic enzymes[34] and nonspecific enzymes[35] (digestive enzymes like pepsin or trypsin), leading to an increase of erosion progression.

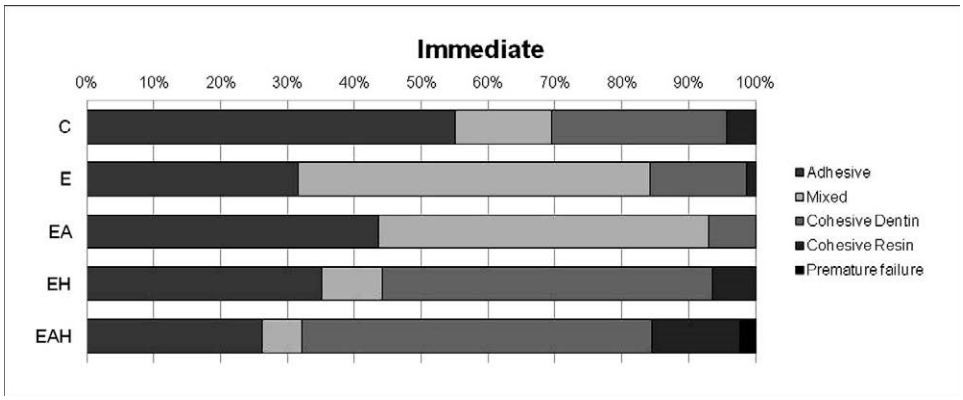

Figure 2. *Bar graph of the failure analysis results for the immediate groups.*

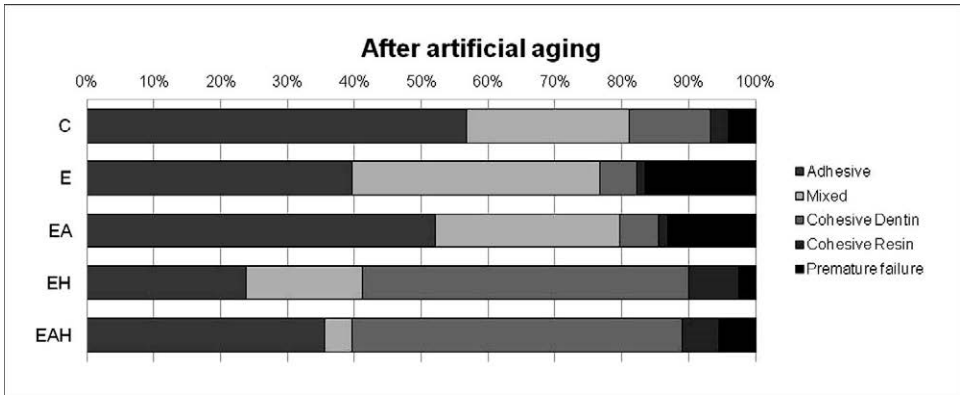

Figure 3. *Bar graph of the failure analysis results for the aged groups.*

Comparing the eroded and eroded/abraded groups, it is noticeable that toothbrushing abrasion in the conditions evaluated had no significant effect on μTBS values. This study was intended to simulate normal conditions, so the specimens were brushed with a 2 N load.[19] Although brushing with forces up to 4 N leads to compression of the organic matrix on the eroded dentin surface,[10] lower forces like those used in this study may not promote significant structural changes in this organic mesh.[9]

There is still some debate about which mode of application (total-etching or self-etching) is the most suitable when using universal adhesives in dentin, but it has been generally found that both techniques promote similar immediate bond strength values.[36-38] However, self-etching may be preferable in long-term *in vitro* studies.[39,40] Nevertheless, this has not been confirmed in clinical studies[41,42] and further long-term studies are necessary.

A previous study comparing the bond strength of a universal adhesive system applied by total-etching and self-etching modes found that the acid etching on sound dentin did not reduce the immediate μTBS values. However, it was observed by transmission electron microscopy that there were areas of low-quality hybridization after the total-etching approach, in particular in the form of a porous and poorly resin-infiltrated collagen mesh.[39] Thus, it was expected that over time the bond strength in the total-etching group would be lower than that of the self-etching group. Indeed, this is in agreement with this study's results, since lower bond strength values were observed after artificial aging in the C, E, and EA groups when the total-etching mode was used.

The inability of adhesive monomers to penetrate the whole depth of the organic matrix exposed by acid etching, associated or not with erosive challenges, creates areas rich in water and poor in adhesive monomers in the hybrid layer.[11] Variations in temperature during thermocycling cause mechanical stresses in these areas due to different coefficients of linear thermal expansion.[22] The stresses can directly induce crack propagation through the bonded interface and may be responsible for the lower μTBS values observed in total-etching and eroded groups. The degradation of the hybrid layer in these groups may be even greater since the phosphoric acid etching[43] and the erosive challenges[34] activate the metalloproteinases and cathepsins from dentin, which can hydrolyze the collagen fibrils and thus reduce the bond strength. A study comparing the bond stability of a universal adhesive by total-

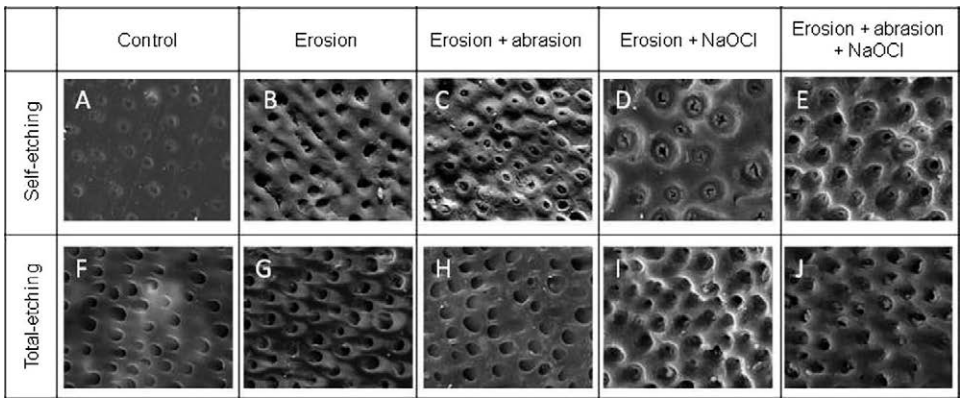

Figure 4. *Scanning electron microscopy images of the different organic matrix conditions when applying the universal adhesive using both adhesive techniques. Note that when using the self-etching mode, the dentinal tubules are occluded by smear layer in the control (A) and opened in the other subgroups (B-E). When using the total-etching mode, the dentinal tubules are opened in all subgroups (F-J). The deproteinized groups (D, E, I, J) present a characteristic funnel shape around the tubules.*

etching and self-etching modes showed that metalloproteinase activation occurs irrespective of the technique employed. However, higher bond strength values and lower nanoleakage have been reported with the self-etch mode.[40]

In deproteinized groups, a higher number of dentin cohesive failures were observed.[44] This may be related to the absence of a hybrid layer and consequent reduction of interfacial defects, which increases the bond strength and makes it closer to the cohesive strength of dentin. After artificial aging, a higher number of premature failures were observed in the E and EA groups, which is probably related to the presence of an increased amount of interfacial defects in these groups compared with the deproteinized ones. This finding reinforces the idea that deproteinization may be a viable alternative to improve not only the bond strength values but also the durability of the adhesion to eroded dentin.

Use of the deproteinization technique on eroded dentin seems to be promising and should be thoroughly investigated. Although some concern exists related to pulp toxicity, the outward dentinal fluid movement has a protective effect against the penetration of substances applied on the dentin surface,[45] reducing the possible cytotoxic effects of sodium hypochlorite. In addition, the remaining dentin thickness acts as a barrier contributing to the cytotoxicity reduction.[46,47] Indeed, this technique has promoted satisfactory clinical results with no significant difference in postoperative sensitivity compared with conventional adhesive technique, with up to 5 years of recall.[48,49]

However, it is important to consider some limitations of the sodium hypochlorite application. First, the stability of this agent is affected by storage conditions, such as temperature, time, and ultraviolet light.[50] Second, it increases the adhesive procedure duration, since 10% sodium hypochlorite must be applied for 1 minute because using lower concentrations of the solution or reduced a time resulted in incomplete collagen removal, leaving denaturated and disorganized organic residues on the surface, thus impairing the adhesive penetration.[51]

The results obtained in this study indicate that sodium hypochlorite is a promising technique to enhance bond strength to eroded dentin. However, it is important to keep in mind that additional clinical studies are needed to evaluate the long-term effect of the organic matrix on adhesion under *in vivo* conditions before adopting this protocol as a restorative therapy for eroded dentin lesions.

CONCLUSION

Considering the limitations of this *in vitro* study, it was concluded that the combined effect of erosion and toothbrushing abrasion did not significantly influence the immediate μTBS values to dentin when using a universal adhesive in total-etching and self-etching modes. Artificial aging negatively affected bond strength to sound, eroded, and eroded/abraded dentin when the total-etching mode was used. The deproteinization technique using 10% sodium hypochlorite increased the μTBS values and maintained the bond stability to eroded and eroded/abraded dentin.

Acknowledgements

The authors thank Professor Ivan Balducci for helping with the statistical analysis and CAPES (Coordination of Training of Higher Education Graduate) for financial support.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local oversight committee guidelines and policies of the Institute of Science and Technology, São Paulo State University.

Conflict of Interest

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

(Accepted 14 August 2017)

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Determination of Caries Lesion Activity: Reflection and Roughness for Characterization of Caries Progression

M Ando • S Shaikh • G Eckert

Clinical Relevance

The results from this study indicate that reflection and roughness of a surface can be used for determination of noncavitated caries lesion progression. As caries lesions progress, surface appearance becomes duller and surface texture rougher.

SUMMARY

Caries lesion progression is difficult to determine with visual and tactile examinations. The hypothesis of this study was that reflection and roughness measurements could determine caries progression. Ground/polished sound human enamel specimens were analyzed at baseline (sound) and after two four-day demineralization periods for reflection using optical reflectometry (ORef) and for roughness using optical surface profilometry (SPro). Specimens were demineralized using a microbial–*Streptococcus mutans* caries model. Comparisons among the periods for ORef and SPro were performed using repeated measures analysis of variance. Two-sample *t*-tests were used for differences in transverse microradiography. The integrated mineral loss and depth of the four-day demineralization period were significantly smaller than those for the eight-day demineralization period ($p<0.01$). With increased demineralization time, reflection was significantly decreased and roughness was significantly increased ($p<0.01$). Correlation between ORef and SPro was moderate ($r=-0.63$). Both reflection and roughness can be characterized for nondestructive longitudinal assessment of caries lesion progression.

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DOI: 10.2341/16-236-L

INTRODUCTION

Noncavitated caries lesion activity can be defined as follows: A lesion that continues to demineralize is described as an active caries lesion, and a lesion that has stopped further demineralization is referred to as an inactive or arrested caries lesion.[1] Since caries can be arrested or reversed at their early stages, it is not sufficient to simply identify the severity of caries lesions. We know some of these lesions will progress

and therefore are in need of some form of intervention, while others may be scars of past damage (inactive/arrested) and therefore will not require any intervention. In order to select an appropriate treatment modality and aid in the clinical decision-making process, caries diagnosis and assessment of caries lesion activity are of critical importance. An incorrect diagnosis will result in incorrect treatment decisions, particularly with respect to irreversible treatments, such as dental restorations.

Visual and tactile examinations are the most common/traditional methods. Prior reports suggest that surface reflection and texture characterize carious lesion activity, with chalky and rough surfaces being active and smooth, shiny, and hard surfaces being inactive.[2-6] Based on this criterion, several studies were conducted. A study[7] showed that the intraexaminer unweighted kappa values for caries activity assessment had a wide range from 0.31 to 0.61 for ICDAS II criteria[8] plus the Lesion Activity Assessment system[5] and from 0.36 to 0.51 for Nyvad criteria.[3] ROC analysis showed that the devised classification system for determining lesion activity had acceptable accuracy (area under curve=0.84).[5] Activity kappa was in the poor to good range.[9] When visual and tactile examination was combined with the information of biofilm/plaque, the intraexaminer agreement (weighted kappa) was 0.61 and sensitivity/specificity were 0.78/0.40.[10] A study indicated that three dental examiners were unable to differentiate between the appearance (visual and tactile) of inactive lesions and active lesions.[11] Another study also showed the inability of experienced examiners to distinguish roughness by tactile examination; although examiners could repeat their own scores, they were not consistent with each other.[12] Although surface reflection and texture characterize the carious lesion activity, previous studies highlight the difficulty in using subjective assessments of caries lesion activity. There are limited reports available, especially with controlled laboratory specimens, on objective measurements of both reflection and roughness in relation to severity of demineralization (lesions). Therefore, there is a need to establish a relationship of the dynamic caries process with objective and quantitative measurements, such as reflectometry, profilometry, and histology. The hypothesis of this study was that enamel demineralization causes diffuse reflection and a rough surface. Longer demineralization times amplify these phenomena when a surface is dried. Therefore, the aim of this study was to determine whether the measurements of tooth surface reflection and roughness could distinguish severity and activity of enamel demineralization objectively.

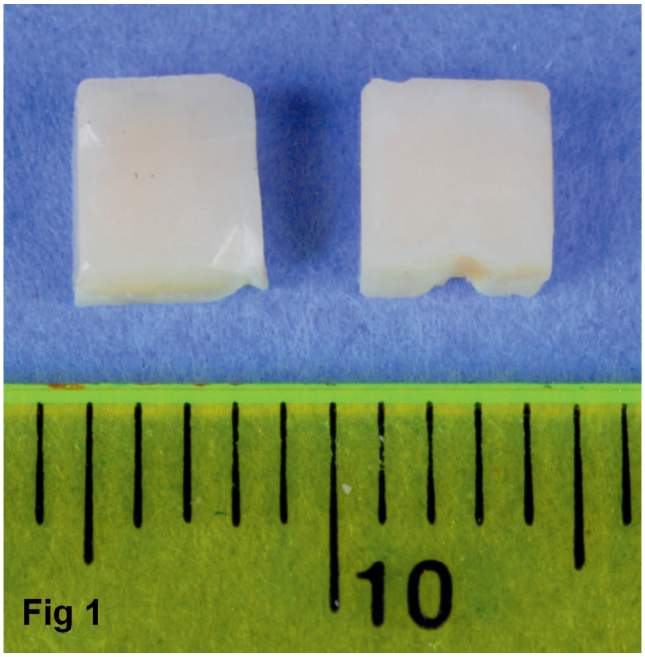


Figure 1. *Image of specimen.*

METHODS AND MATERIALS

Specimen Preparation

Extracted human teeth were collected from dental practitioners in the state of Indiana and transported in 0.1% thymol solution to the Oral Health Research Institute, Indiana University School of Dentistry. The collection of human teeth for use in dental laboratory research studies has been approved by the Indiana University Institutional Review Board. Sound permanent incisors were sterilized with ethylene oxide gas. From these teeth, 25 3 × 3 × 2-mm blocks were prepared (Figure 1). First, the enamel side was ground to establish a flat surface, and the secondary dentin side was ground by RotoForce-4 and RotoPol-31 (Struers Inc, Rødovre, Denmark). The exposed 3 × 3-mm enamel surface was polished by RotoForce-4 and RotoPol-31 until the total height was 2.0 mm (2000 μm). On the dentin side, notches were made for orientation of the specimen. While the specimens were not in use, they were kept in a humid and cold environment (4°C).

Characterization of Sound Enamel

Optical Reflectometry (ORef)—Specimens were placed inside the slot of a custom-made specimen holder. Reflectivity was measured with a computer-

guided optical spectrometer configured as a reflectometer (AvaSoft version 7.1.0 Full, Avantes Inc, Broomfield, CO, USA). The system was calibrated using a ground and polished human incisor tooth, with sound enamel as reference material prior to analysis. This system consists of a fiber-optic spectrometer (AvaSpec-2048, Avantes) that measures in the 200- to 1100-nm range and a 2048-pixel CCD detector. It also consists of a tungsten halogen light source (AvaLight-HAL, Avantes), which has a wavelength range of 360 to 2000 nm and optical power of 0.5 mW with 200-μm fiber. A fiber-optic reflection probe (FCR-7UV100-2-1.5×100, Avantes) was placed perpendicular to the specimen surface using a special angled fiber holder (AFH-15, Avantes). This fiber-optic reflection probe has six illuminations around one read of 200-μm fibers and a usable transmission wavelength range of 200 to 1100 nm. The reflectivity was obtained in the 380- to 780-nm range. This range was chosen based on the preliminary data that showed negligible fluctuation of sound-surface reflection. Average reflectance (%) was determined for each specimen.

Optical Surface Profilometry (SPro)—All specimens were subjected to scanning with computer-guided optical profilometry (Proscan 2000A, Scantron Industrial Products Ltd, Taunton, UK). This uses 50 W of halogen as a light source. The light is focused onto the measurement surface as a spot approximately 4 μm in diameter. The scanning frequency was 100 Hz, with a step size of 0.01 mm for the X-axis and of 0.01 mm for the Y-axis and a resolution of 0.01 μm. The center of the surface (scan area: 1.0×1.0 mm) was scanned for each specimen. The profilometric data were digitally recorded and processed by dedicated software (Proscan 2000 version 2.0.17). In this study, the parameters were limited to arithmetical mean roughness, R_a (μm), which is the most commonly used surface roughness parameter, defined as the mean deviation of the profile from the centerline, where the centerline (sometimes called mean reference line) is derived from the profile by filtering out its short-wavelength components. Ten profiles were recorded for each scan area, and mean values for each specimen were obtained by averaging them.

Demineralization

The specimens were demineralized by placing them in an *in vitro*, microbial caries model.[13] The specimens were inoculated with a mid–log phase culture of *Streptococcus mutans* A32-2 (absorbance of 0.5 at 540 nm) in trypticase soy broth without dextrose, supplemented with 5% sucrose (TSBS). After implantation, the specimens were placed in a caries-forming vessel and exposed to circulating TSBS solution for 30 minutes three times per day and to circulating mineral wash solution for 22.5 hours per day. The circulating fluids were delivered and removed from the treatment vessel by a peristaltic pump regulated by a timer. The specimens were treated for a total of eight days. The ORef and SPro data were obtained at day 4 and day 8 of bacterial demineralization, respectively. Based on a pilot study (unpublished) and previous studies,[14,15] four days and eight days of demineralization were chosen. Specimens were sterilized with ethylene oxide gas in between demineralization prior to bacterial exposure.

Characterization of Demineralized Enamel

The ORef and SPro data were obtained at four days and eight days of bacterial demineralization as described previously. At the end of each demineralization, five specimens were utilized for transverse microradiography (TMR) analysis for model validation.

Model Validation

Depth and mineral loss of specimens were determined by TMR. One section (100 μm thick) was cut from each selected specimen through the center of the specimen. All sections were mounted together with an aluminum step wedge on a glass plate (High Resolution UF Plate, Microchrome Technology, Inc, San Jose, CA, USA) and exposed to Cu(Kα) X-rays at 20 kV and 30 mA for 65 minutes. The glass plates were developed accordingly. The plates of TMR were examined with a customized computer program (Transverse Micro-radiography, Inspektor Research Systems BV, Amsterdam, Netherlands). The following data were recorded for each section: 1) depth (D [μm]) of each demineralized enamel to 83% mineral (95% of mineral in sound enamel) and 2) difference in mineral composition between remineralized and sound enamel (integrated mineral loss [IML] or ΔZ [vol%×μm]).

Data Analysis

Comparisons among the sound and demineralization times for differences in ORef and SPro were performed using repeated measures analysis of variance (ANOVA). Comparisons among the demineralization times for differences in TMR IML and lesion depth were performed using one-way ANOVA. The ranks of the measurements were used in the

Table 1: *Average and Standard Deviation of Transverse Microradiography*

Demineralization Time (Days)	Depth (μm)	Integrated Mineral Loss (vol%×μm)
4	16.1 ± 5.3	310 ± 154
8	30.7 ± 3.8	950 ± 159

analysis. Spearman (nonparametric) correlation coefficients were calculated to evaluate the associations between measurements. The correlation calculations treated each time point as independent data, ignoring the within-specimen correlations over time.

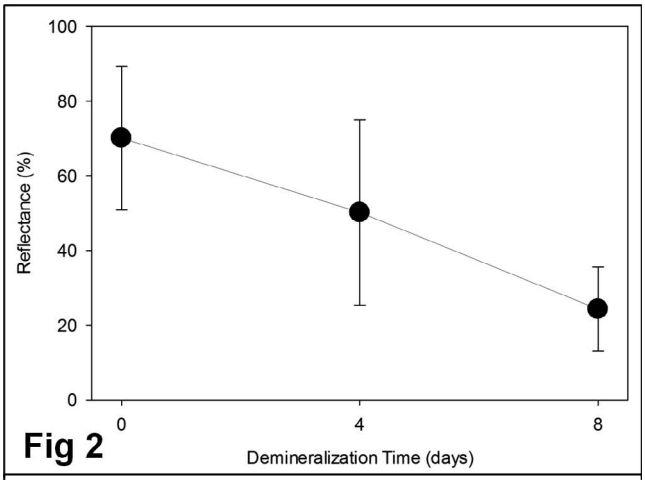
RESULTS

The results of TMR indicate that the IML and depth of four-day demineralization were significantly less than those of eight-day demineralization ($p<0.01$, Table 1). With increased demineralization time, reflection was significantly ($p<0.01$) decreased (Figure 2), and roughness was significantly increased (Figure 3). Correlation between ORef and SPro was moderate ($r=-0.63$). TMR parameters showed weak correlation with ORef ($r=-0.26$ for IML and $r=-0.13$ for depth) and moderate correlation with SPro ($r=0.67$ for IML and $r=0.55$ for depth).

DISCUSSION

Our longitudinal data confirmed that objective reflection and roughness measurements can be used for determination of caries lesion activity, especially for caries progression. With increased demineralization time, reflection was significantly decreased, and roughness was significantly increased. That means that as caries lesions progress, surfaces appear to be dull and get rough. The presence of dull and rough lesions highlights the probability of caries lesion activity when examined by clinicians.

A proof-of-concept study was conducted to demonstrate whether reflection could be used to determine caries lesion activity.[16] Reflection was measured by the vertical reflection intensity using extracted teeth that had smooth surface caries lesions. Caries activity was assessed by Nyvad criteria.[3] The results indicated low-intensity values for active lesions and higher values for inactive lesions. Active lesion surfaces presented higher roughness values than inactive lesion surfaces. Also, active lesions had larger porosity than inactive lesions. Another study was performed with natural noncavitated caries lesions. Cross-sectional data indicated that reflection may be able to be used for determination of caries



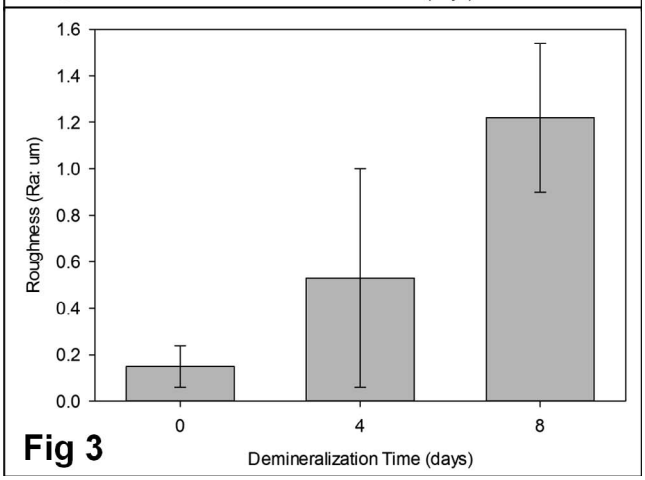


Figure 2. *Average and standard deviation of reflectance.*
Figure 3. *Average and standard deviation of roughness.*

activity.[17] Extracted teeth that had noncavitated lesions on smooth surfaces were used in this study. Caries activity was visually assessed by Nyvad criteria without probe.[3] Active lesions presented lower reflection values than inactive lesions and sound enamel surfaces. Also, active lesions presented higher roughness values than inactive and sound surfaces. A limitation of these studies was their cross-sectional design. Also, one of the studies used a total of only two specimens, one for active and one for inactive status. Advantages of our current study are utilization of a controlled microbial demineralization model and histological validation. Progression of demineralization was confirmed by histology. Despite differences between previous studies and this current study, our *in vitro* longitudinal results demonstrated the same trend to confirm these findings; as lesions progressed, reflection was decreased/lower, and roughness was increased/higher.

Sound/intact surfaces should be smoother than demineralized surfaces that have microchannels. When light rays strike a smooth surface, the reflected rays are parallel to each other. This is known as specular reflection, and surfaces that cause specular reflection appear shiny.[18] This may be the reason why sound (0-hour demineralization) reflection values were higher and roughness values were smaller. Based on chemical analysis and histopathological observations, the initial stage of caries development is characterized by the opening of the intercrystalline spaces without the destruction of the surface and subsequent creation of microchannels.[19-21] Then acid penetrates from the surface into the subsurface enamel through microchannels,[19,22,23] resulting from the dissolution of the subsurface mineral. Microchannels allow the diffusion of saliva, water, and acid into the lesion body. As these microchannels enlarge, bacteria may also gain access. These microchannels are found to be about 0.5 to 1.5 μm in width in artificial lesions[19] and range from 0.2 to 1.0 μm in width in early natural enamel lesions.[24] The development of microchannels results in an irregular surface. The irregular surface reflects the light rays in various directions. This causes the surface to appear dull (nonglossy)[18] as seen in active caries lesions. Also, irregular surfaces create rough surfaces. A study using scanning electron microscopy indicated that microchannels developed at caries initiation and increased in size with continued demineralization time.[25] These may be reasons that explain that as lesions progressed, demineralized enamel showed decreased/lower reflection and increased/higher roughness compared to sound surfaces.

The results from our current study may suggest development of a handheld instrument for an objective and quantitative means to measure caries lesion activity at the time of examination (chair side). When successful, this instrument can be of great significance for clinical decision making in the management of dental caries. Particularly, both reflection and roughness measurements can be translated to clinicians with an objective and quantitative measurement for determination of caries lesion activity at the time of examination. Activity is what should drive caries interventions; therefore, objective measurement of activity will greatly facilitate clinical decision making for more effective caries management.

CONCLUSIONS

As teeth undergo continued demineralization, reflection is progressively decreased and roughness progressively increased. Both decreasing reflection and increasing roughness can be characterized for caries lesion progression. The increase in dullness and roughness of surfaces would potentially point toward active caries progression.

Regulatory Statement

This study was conducted in accordance with all the provisions, guidelines, and policies of the Indiana University Institutional Review Board. The approval code for this study is NS0911-07.

Conflict of Interest

The authors confirm that there are no known conflicts of interest associated with this publication. Also, there has been no financial support for this work that might have influenced its outcome.

(Accepted 14 March 2017)

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Viscoelastic Properties of Contemporary Bulk-fill Restoratives: A Dynamic-mechanical Analysis

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Clinical Relevance

Viscoelastic properties of bulk-fill restoratives varied between materials and were environment dependent. Resin-coating of reinforced bulk-fill glass ionomers does not positively influence elastic properties.

SUMMARY

This study investigated the viscoelastic properties of contemporary bulk-fill restoratives in distilled water and artificial saliva using dynamic mechanical analysis. The materials evaluated included a conventional composite (Filtek Z350), two bulk-fill composites (Filtek Bulk-fill and Tetric N Ceram), a bulk-fill giomer (Beautifil-Bulk Restorative), and two novel reinforced glass ionomer cements (Zirconomer [ZR] and Equia Forte [EQ]). The glass ionomer materials were also assessed with and without resin coating (Equia Forte Coat). Test specimens 12 × 2 × 2 mm of the various materials were fabricated using customized stainless-steel molds. After light polymerization/initial set, the specimens were removed from the molds, finished, measured, and conditioned in distilled water or artificial saliva at 37°C for seven days. The materials (n=10) were then subjected to dynamic mechanical testing in flexure mode at 37°C and a frequency of 0.1 to 10 Hz. Storage modulus, loss modulus, and loss tangent data were subjected to normality testing and statistical analysis using one-way analysis of variance/Dunnett's test and *t*-test at a significance level of $p < 0.05$. Mean storage modulus ranged from 3.16 ± 0.25 to 8.98 ± 0.44 GPa, while mean loss modulus ranged from 0.24 ± 0.03 to 0.65 ± 0.12 GPa for distilled water and artificial saliva. Values for loss

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DOI: 10.2341/16-365-L

tangent ranged from 45.7 ± 7.33 to 134.2 ± 12.36 (10^{-3}). Significant differences in storage/loss modulus and loss tangent were observed between the various bulk-fill restoratives and two conditioning mediums. Storage modulus was significantly improved when EQ and ZR was not coated with resin.

INTRODUCTION

Due to the declining popularity of amalgam, the pursuit of "tooth-colored alternatives" has intensified over the past few years.[1] Composite resins, glass ionomer cements, and hybrids of these materials are constantly being enhanced to improve their clinical handling and performance.[2,3] Innovative bulk-fill composites were introduced to address the need for incremental material placement arising from limited depth of cure and polymerization shrinkage associated with conventional composites.[4] The incremental technique also has several disadvantages, including the incorporation of voids or contamination between layers and placement difficulty in cavities with limited access, and is clinically time consuming to perform. Bulk-fill composites can be placed in increments of 4 mm and are reported to possess enhanced curing and controlled shrinkage.[5] The early moisture sensitivity and low physicomechanical properties of glass ionomer cements have been alleviated by fast-setting, highly viscous and reinforced glass ionomers.[3] Collectively, bulk-fill composite and glass ionomer restoratives simplify clinical procedures and reduce technique sensitivity, chair time, and stress for both dentists and patients, especially when multiple posterior restorations are required.

Posterior direct tooth-colored restorative material should have adequate strength to resist masticatory and occlusal forces. Tooth-colored restoratives were traditionally evaluated using destructive static compression, tension, or flexure tests. These tests, however, only emphasize the elastic component of materials and provide single-event strength values.[6] Dynamic methods are now commonly employed to assess mechanical properties of viscoelastic materials in materials science. Dynamic mechanical analysis (DMA) is particularly well suited for viscoelastic materials, such as composites and glass ionomers, as it can determine both elastic and viscous responses of materials.[7] The test is also able to mimic cyclic masticatory loading that materials are subjected to intraorally.[8] The nondestructive nature of this test allows for the reexamination of specimens after being subjected to different treatments. In addition, a wide range of frequency, temperature, and/or amplitude variations is admissible with DMA. DMA and other dynamic tests are superior to static tests, as they provide greater sensitivity to both macroscopic and molecular relaxation.[9]

The physical properties of tooth-colored restorations are affected by their surrounding chemical environment.[10] Direct tooth-colored restoratives have been shown to leach filler and other constituents when stored in distilled water.[11] As direct tooth-colored restoratives are constantly being surrounded by saliva, findings obtained from storage in distilled water may be of little clinical relevance.[12] The use of artificial saliva allows for better simulation of the way restoratives interact with human saliva.[13] Leaching of restorative constituents has been reported to be higher in artificial salvia when compared to distilled water.[14]

Studies investigating the viscoelastic properties of bulk-fill tooth-colored restoratives using DMA in different conditioning mediums are still lacking. In addition, no research had been done on novel bulk-fill giomer and reinforced glass ionomer restoratives. Giomers, also known as PRG composites, are based on prereacted glass ionomer (PRG) technology in which acid-reactive fluoride-containing glass is reacted with polyacids in the presence of water, freeze-dried, milled, silanized, ground, and used as fillers. Besides fluoride release and tooth demineralization inhibition, giomers also possess antiplaque formation properties.[15-18] Zirconomer and Equia Forte are two recently introduced bulk-fill reinforced highly viscous glass ionomer cements. While Zirconomer is reinforced with nanozirconia fillers, Equia Forte is reinforced with ultrafine, highly reactive glass particles forming a glass "hybrid" restorative. Together with the application of a multifunctional monomer layer, "microlaminate" restorations with improved physical and esthetic properties are achieved. A prospective six-year clinical trial using the "microlamination" technique proved the reliability of this restorative approach.[19] Both bulk-fill reinforced glass ionomers have been promoted as amalgam alternatives.

The objectives of this study were to compare the viscoelastic properties of contemporary bulk-fill restorative materials. Variations in storage and loss modulus as well as loss tangent after conditioning in distilled water and artificial saliva were also compared. For the reinforced glass ionomer cements, the effects of resin coating on viscoelastic properties were also evaluated. The null hypotheses were that there were no differences in viscoelastic behavior

Table 1: *Technical Profiles and Manufacturers of the Materials Evaluated*

Material (Abbreviation)	Manufacturer	Type and Method of Curing	Resin/Liquid	Filler/Powder	Filler Content % by Weight/ % by Volume
Filtek Z350 (ZT)	3M ESPE (St Paul, MN, USA)	Nanohybrid composite (light cured)	Bis-GMA Bis-EMA UDMA TEGDMA	Zirconia/silica cluster, silica nanoparticle	78.5/63.3
Filtek Bulk-Fill (FB)	3M ESPE	Bulk-fill composite (light cured)	Bis-GMA Bis-EMA UDMA Proctylat resins	Zirconia/silica cluster, ytterbium trifluoride	76.5/58.4
Tetric N Ceram Bulk-Fill (TC)	Ivoclar, Vivadent Inc (Amherst, NY, USA)	Bulk-fill composite (light cured)	Bis-GMA Bis-EMA UDMA	Barium glass filler, ytterbium fluoride, spherical mixed oxide	77/55
Beautifil-Bulk Restorative (BB)	Shofu Inc (Kyoto, Japan)	Bulk-fill giomer (light cured)	Bis-GMA UDMA Bis-MPEPP TEGDMA	S-PRG based on F-Br-Al-Si glass	87/74.5
Zirconomer (ZR)	Shofu	Zirconia/reinforced glass ionomer (chemically cured)	Polyacrylic acid solution, tartaric acid	Fluoroaluminosilicate glass, zirconia oxide, pigments, others	Not applicable
GC Equia Forte (EQ)	GC Industrial Co (Tokyo, Japan)	Bulk-fill glass ionomer (chemically cured)	—	Fluoroaluminosilicate glass, polyacrylic acid powder, surface-treated glass	Not applicable
GC Equia Forte Coat (C)	GC Industrial	Nanofilled resin (light cured)	—	Nanofiller	Not available

Abbreviations: Bis-EMA, ethoxylated bisphenol-A-glycidyl methacrylate; Bis-GMA, bisphenol-A glycidyl methacrylate; Bis-MPEPP, bisphenol-A polyethoxy-dimethacrylate; S-PRG, surface-modified prereacted glass; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

between the various restoratives, conditioning in distilled water, and artificial saliva as well as between resin and non–resin-coated glass ionomers.

METHODS AND MATERIALS

Materials selected for this study included a conventional composite (Filtek Z350 [ZT]), two bulk-fill composites (Filtek Bulk-fill [FB] and Tetric N Ceram [TC]), a bulk-fill giomer (Beautifil-Bulk Restorative [BB]), and two reinforced bulk-fill glass ionomer restoratives (Zirconomer [ZR] and Equia Forte [EQ]). The glass ionomer materials were also assessed with and without a nanofilled resin coating (Equia Forte Coat [C]). Details of the materials used and their technical profiles are shown in Table 1. Test specimens $12 \times 2 \times 2$ mm of the various materials were fabricated using customized stainless-steel molds. The materials were mixed according to the manufacturers' instructions where applicable and placed in a single increment into the molds. Excess material was removed by compressing the molds between two Mylar strips with glass slides. The top and bottom surfaces of composite and giomer specimens were subsequently light polymerized with two overlapping irradiation cycles of 10 seconds each using an LED curing light (Demi Plus, Kerr Corp, Orange, CA, USA) with an irradiance of 1330 mW/cm^2. These restoratives were light polymerized for an additional 10 seconds after removal from their molds. For the glass ionomers, specimens were allowed to set for five minutes before removal from their molds. The specimens were carefully finished using fine contouring/polishing discs (Sof-Lex, 3M ESPE, St Paul, MN, USA). For the resin-coated glass ionomer groups, Equia Forte Coat was applied to the test specimens on all four surfaces and light polymerized in two overlapping irradiation cycles of 10 seconds per surface. All specimens were subsequently measured with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan) to ensure standardized specimens with parallel opposing surfaces.

The specimens were then randomly divided into two groups (n=10) and conditioned in either distilled water or artificial saliva at 37°C for seven days. Composition of the artificial saliva used (SAGF medium[20]) is shown in Table 2. The pH of the artificial saliva was checked with a digital pH meter (pH 2700, Eutech, Singapore) and adjusted to 6.8. Both conditioning mediums were replaced every two days to minimize changes in pH over time. Speci-

Table 2: *Composition of the SAGF Medium*

Components	Concentration (mg L^{-1})
NaCl	125.6
KCl	963.9
KSCN	189.2
KH_2PO_4	654.5
Urea	200.0
$NaSO_4 \bullet 10H_2O$	763.2
NH_4Cl	178.0
$CaCl_2 \bullet 2H_2O$	227.8
$NaHCO_3$	630.8

mens were subjected to dynamic mechanical testing (DMA RSA-G2, TA Instruments, New Castle, DE, USA) in distilled water or artificial saliva and flexure three-point bending mode at 37°C with a frequency of 0.1 to 10 Hz. The distance between the supports was fixed at 10 mm, and an axial load of 5 N was employed. Storage modulus, loss modulus, and loss tangent values were obtained for the various bulk-fill restoratives.

Statistical analysis was performed with the SPSS software (version 12.0.1, SPSS Inc, Chicago, IL, USA). Data were checked for normality using the Kolmogorov-Smirnov and Shapiro-Wilk test. Comparisons between materials were performed using one-way analysis of variance and Dunnett's test, while the effects of conditioning medium and resin-coating was appraised using an independent sample *t*-test at a significance level $\alpha = 0.05$.

RESULTS

Mean storage modulus, loss modulus, and loss tangent for the various materials and mediums are shown in Tables 3 through 5. Data were found to be normal, and parametric data analysis was permissible. One-way analysis of variance indicated significant differences in viscoelastic behaviors between bulk-fill materials in both distilled water and artificial saliva. Mean storage modulus ranged from 3.19 ± 0.30 to 7.44 ± 0.28 GPa in distilled water and 3.16 ± 0.25 to 8.98 ± 0.44 GPa in artificial saliva (Table 3). For both mediums, the highest storage modulus was observed with EQ and the lowest with ZRC. With the exception of EQ, storage modulus of the composite and giomer restoratives was generally higher than that of EQC, ZR, and ZRC in both conditioning mediums. Significant differences in storage modulus were observed between conditioning in distilled water and artificial saliva for FB, TC, BB, EQ, and EQC. Storage modulus of FB, BB, and EQ was significantly larger after conditioning in artificial saliva. Uncoated glass ionomer specimens had a significantly higher storage modulus than their resin-coated counterparts when conditioned in both distilled water and artificial saliva.

Table 3: *Mean Storage Modulus Values (GPa) of the Various Materials (Standard Deviations in Parentheses)*[a]

Materials (Code)	Distilled Water	Artificial Saliva
Filtek ZT (ZT)	5.76 (0.42) A	5.48 (0.57) A
Filtek Bulk-Fill (FB)[b]	5.48 (0.45) AB	6.17 (0.70) A
Tetric N Ceram (TC)[b]	4.77 (0.54) BC	3.63 (0.37) B
Beautifil (BB)[b]	5.27 (0.62) AC	5.91 (0.56) A
Equia Forte without resin coat (EQ)[b]	7.44 (0.28)	8.98 (0.44)
Equia Forte with resin coat (EQC)[b]	4.53 (0.16) C	4.16 (0.27)
Zirconomer without resin coat (ZR)	3.75 (0.36)	3.60 (0.35) B
Zirconomer with resin coat (ZRC)	3.19 (0.30)	3.16 (0.25) B

[a] *Values with same letters in the same column are not significantly different.*
[b] *Indicates significant differences between distilled water and artificial saliva.*

Mean loss modulus ranged from 0.24 ± 0.03 to 0.65 ± 0.12 GPa in distilled water and 0.24 ± 0.03 to 0.51 ± 0.09 GPa in artificial saliva (Table 4). TC and FB had the highest loss modulus after exposure to distilled water and artificial saliva, respectively. For both mediums, the lowest loss modulus was observed with ZRC. When conditioned in distilled water, loss modulus of the composite and giomer restoratives was significantly greater than the glass ionomer materials. The same trend was generally observed after conditioning in artificial saliva with the exception of EQ. Significant differences in loss modulus between conditioning in distilled water and artificial saliva were observed for TC, EQ, and EQC. Loss modulus of TC was about 50% lower when exposed to artificial saliva. Unlike TC and EQC, storage in artificial saliva produced higher loss modulus for EQ. For both glass ionomers, resin coating generally resulted in significantly lower loss modulus in artificial saliva.

Loss tangent values of the restoratives ranged from 45.7 ± 7.33 to 134.2 ± 12.36 (10^{-3}) in distilled water and 53.7 ± 5.70 to 92.5 ± 9.50 (10^{-3}) in artificial saliva (Table 5). For both mediums, the greatest loss tangent was observed with TC and the lowest with EQ. Loss tangent values of the composite and giomer restoratives were higher than the glass ionomer restoratives after conditioning in distilled water. Such trends were not observed in artificial

Table 4: *Mean Loss Modulus Values (GPa) of the Various Materials (Standard Deviations in Parentheses)*[a]

Materials (Code)	Distilled Water	Artificial Saliva
Filtek ZT (ZT)	0.47 (0.05) A	0.47 (0.06) A
Filtek Bulk-Fill (FB)	0.45 (0.05) A	0.51 (0.09) A
Tetric N Ceram (TC)[b]	0.65 (0.12)	0.34 (0.06) B
Beautifil (BB)	0.47 (0.08) A	0.44 (0.06) A
Equia Forte without resin coat (EQ)[b]	0.34 (0.06) BC	0.48 (0.05) A
Equia Forte with resin coat (EQC)[b]	0.35 (0.02) B	0.31 (0.03) B
Zirconomer without resin coat (ZR)	0.27 (0.03) CD	0.25 (0.03) C
Zirconomer with resin coat (ZRC)	0.24 (0.03) D	0.24 (0.03) C

[a] *Values with same letters in the same column are not significantly different.*
[b] *Indicates significant differences between distilled water and artificial saliva.*

Table 5: *Mean Loss Tangent Values (10^{-3}) of the Various Materials (Standard Deviations in Parentheses).*[a]

Materials (Code)	Distilled Water	Artificial Saliva
Filtek ZT (ZT)	82.7 (5.25) AB	77.3 (24.8) ABCD
Filtek Bulk-Fill (FB)	83.9 (5.97) AB	84.2 (10.41) AB
Tetric N Ceram (TC)[b]	134.2 (12.36)	92.5 (9.50) A
Beautifil (BB)[b]	89.1 (5.76) A	74.7 (5.89) BC
Equia Forte without resin coat (EQ)	45.7 (7.33)	53.7 (5.70) D
Equia Forte with resin coat (EQC)	79.5 (5.87) BC	76.0 (4.06) BC
Zirconomer without resin coat (ZR)	72.9 (3.25) C	70.6 (3.98) C
Zirconomer with resin coat (ZRC)	74.7 (4.55) C	77.4 (7.77) BC

[a] *Values with same letters in the same column are not significantly different.*
[b] *Indicates significant differences between distilled water and artificial saliva.*

saliva. Significant differences in loss tangent values were observed between mediums for TC and BB. Both these materials exhibited significantly greater loss tangent after exposure to distilled water. While ZR showed no significant difference with resin coating, loss tangent values were significantly greater for EQC in both distilled water and artificial saliva.

DISCUSSION

The viscoelastic properties of contemporary bulk-fill restoratives in distilled water and artificial saliva using DMA were studied. As viscoelastic properties were found to be material and conditioning medium dependent, the null hypotheses were rejected. With DMA, dynamic testing can be performed with a range of temperature, frequency, and amplitude modifications. Temperature was fixed at body temperature (ie, 37°C), while frequency was set at 0.1 to 10 Hz to represent a range from close to "static" testing (0.1 Hz) to the upper limit of normal chewing frequency.[21] Dimensions for the flexure specimens were based on the work of Yap and others.[22] Significant and positive correlations were observed for both flexural strength and modulus between the miniflexural specimens and their lengthier International Organization for Standardization counterparts (25×2×2 mm).[22] Besides being clinically more relevant, the miniflexural specimens are also easier to fabricate and required less material. SAGF medium was used, as its pH, buffering capacity, content, and viscosity mimicked that of natural salvia and has been reported to allow for specification of fluoride release and corrosion behavior of dental biomaterials.[20]

Storage modulus represents the rigidity or stiffness of the restoratives, while loss modulus indicates their ability to flow. None of the restoratives evaluated had similar or higher modulus than dentin, which is approximately 18 GPa.[23] For both conditioning mediums, EQ was significantly more rigid and will deform less than the other materials under functional stresses, supporting its indication for posterior restorations. This corroborated a recent systematic review that reported comparable failure rate between highly viscous glass ionomers and amalgam in permanent posterior teeth.[24] The zirconia-reinforced glass ionomer, however, had the lowest storage modulus regardless of resin coating. This may be attributed to the lack of chemical adhesion between the zirconia fillers and the polysalt matrix, resulting in areas of stress concentrations.[25] Apart from EQ, the composite and giomer materials were generally significantly stiffer than EQC, ZR, and ZRC. Mesquita and others[7] reported an association between storage modulus and filler weight content. Even with the apparently high percentage of fillers by weight of TC, its storage modulus was still significantly lower than the other polymeric materials. This was due to TC's low filler volume (notwithstanding its high filler weight), reiterating the greater importance of percentage filler volume in composite characterization. Restoratives with lower modulus have higher elastic deformation when loaded, leading to possible disruption of restoration–tooth interfacial bonding that is associated with postoperative sensitivity, microleakage, and recurrent caries.[26] In both distilled water and artificial saliva, the loss modulus of the composites and giomer was mostly higher than the glass ionomer materials. The polymeric materials thus flowed more than the glass

ionomers when subjected to functional loading. Ranking of loss modulus between polymeric materials differed between conditioning mediums. Viscous flow may help reduce or delay fracture, wear, and debonding of restorations.[27] Materials with high loss modulus can, however, present with small permanent dimensional changes that may be clinically pertinent.

Loss tangent expresses the energy dissipation capacity of the restoratives and is determined by the ratio of the loss modulus to storage modulus. Mechanical energy is dissipated through conversion into heat by molecular motion and is associated with unrecoverable viscous loss.[28] Friction between filler particles and the polymer matrix had been suggested as an important source of energy dissipation during deformation under stress.[29] The lower the loss tangent, the quicker the restorative will respond to load and return to its original shape.[7] In both conditioning mediums, loss tangent values of EQ were significantly lower than the other materials evaluated. Findings were consistent with those of Helvatjoglu-Antoniades and others,[30] who reported that composites with the highest filler content and highly viscous glass ionomer exhibited the highest storage modulus and lowest loss tangents. As the same authors also report significant variation of viscoelastic properties with temperature, temperature variations will be taken into consideration for future studies. The small loss tangent values obtained indicate that the restoratives evaluated have a modest viscous component over the frequency range applied, indicating that that they were more "elastic-like" in nature.

In the present study, the restoratives were conditioned for seven days and tested in distilled water or artificial saliva at 37°C. Significant differences between conditioning mediums were property and material dependent. For storage modulus, significant differences were observed for all materials with exception of the conventional composite (ZT) and zirconia reinforced glass ionomer (ZR and ZRC). Significant differences in loss modulus were observed between mediums for the bulk-fill composite TC and glass "hybrid" restorative (EQ and EQC), while loss tangent values were significantly different for TC and the bulk-fill giomer BB. While conditioning in distilled water resulted in better viscoelastic properties for some materials, it reduced storage and loss modulus for others when compared to conditioning in artificial saliva. The interactions between distilled water/artificial saliva and the restoratives are highly complex. For the composite and giomer materials, water sorption from both mediums can result in plasticization and degradation. Absorption of water molecules causes expansion, increasing effective free volume and ease of polymer chain movements, affecting both storage and loss modulus.[30,31] Degradation from the leaching of fillers and unreacted monomers may be higher in artificial saliva than in distilled water[14] and is anticipated to influence viscoelastic properties. Due to the varied outcomes, the conditioning medium of choice remains equivocal and warrants further investigations.[32]

Glass ionomer cements consist of basic fluoroaluminosilicate glasses and acidic copolymers that set chemically by acid–base reactions. Water is the reaction medium into which cement-forming cations are leached and transported to react with polyacids. It also serves to hydrate the cross-link matrix, increasing the cement strength. The final set glass ionomer structure contains a substantial amount of unreacted glass that acts as fillers for the set cement.[33] While previous generations of glass ionomer cements were susceptible to early moisture sensitivity, more recent fast-set and resin-modified cements have improved moisture tolerance. Resin coating is, however, still advocated to improve physicomechanical properties and clinical longevity of highly viscous glass ionomer cements.[19,34,35] For both EQ and ZR, resin coating did not positively affect their viscoelastic properties. Storage modulus was superior when specimens were not resin coated. For EQ, storage modulus was 1.64 and 2.16 times greater in distilled water and artificial saliva, respectively, when resin coating was omitted. The higher storage modulus observed with conditioning in artificial saliva may be contributed in part by its phosphate content.[36] Loss modulus was also generally better when the reinforced glass ionomers were not resin coated. The difference in loss modulus was, however, discrete when compared to storage modulus. Loss tangent was correspondingly lower for EQ without resin coating. The current data supported those of prior studies. Wang and others[37] reported that early water exposure did not weaken highly viscous glass ionomer materials, while Pilo and others[38] concluded that there was no need to protect highly viscous glass ionomers from water to improve strength. Other investigators have, however, reported better physicomechanical properties with resin coating.[34,39,40] The apparent incongruities could be ascribed to differences in glass ionomers/resin coatings evaluated as well as variances in physicomechanical properties assessed, testing methodologies, and protocols. Further static and dynamic testing as well as clinical trials are warranted before a definitive inference can be made.

CONCLUSIONS

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

1) Viscoelastic properties were found to be material dependent. With the exception of the glass "hybrid" restorative (EQ), the composite and giomer materials generally had higher storage and loss modulus than the reinforced glass ionomer cements.
2) As the viscoelastic properties of the giomer bulk-fill restorative were comparable or superior to composites, they could be indicated for posterior restorations.
3) Significant differences in viscoelastic properties were observed between conditioning in distilled water and artificial saliva. The variations were again material dependent.
4) Resin coating did not positively affect viscoelastic properties and is not required to improve elastic properties.

Acknowledgements

This work was supported by research grant RG531-13HTM, Faculty of Dentistry, University of Malaya. The authors would like to thank 3M EPSE, Shofu Asia, GC Asia, and Ivoclar Vivadent Inc for their material support.

Conflict of Interest

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

(Accepted 27 March 2017)

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Monolithic Ceramics: Effect of Finishing Techniques on Surface Properties, Bacterial Adhesion and Cell Viability

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Clinical Relevance

Clinical procedures should be performed with caution considering that rough surfaces are directly related to higher bacteria adhesion. However, a polishing procedure may lead to a temporary inflammatory tissue reaction.

SUMMARY

Introduction: This study evaluated the morphology, biofilm formation, and viability of human gingival fibroblasts in contact with two monolithic ceramics after two different finishing techniques: polishing or glazing. For this, 92 blocks (4.5 × 4.5 × 1.5 mm) of each ceramic were made using high translucency zirconia partially stabilized by yttrium (YZHT) and lithium silicate reinforced by zirconium (ZLS).

Methods and Materials: Blocks were sintered and then divided into glazing (g) or polishing (p) surface finish. Surface roughness (Ra and RSm) was evaluated through a contact rugosimeter and profilometry. Specimens were contaminated for heterotypic biofilm formation with *Streptococcus mutans*, *Streptococcus*

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DOI: 10.2341/17-011-L

***sanguinis* and *Candida albicans* for 16 hours. Biofilm was quantified by counting the colony forming units (CFU/mL) and analyzed by scanning electron microscopy (SEM). Fibroblast viability was evaluated by MTT assay. Surface free energy (SFE) was also determined. Roughness data were evaluated using nonparametric tests, while SFE, MTT and CFU results were evaluated by analysis of variance and Tukey test, and MTT data were also submitted to *t*-test (all, α=0.05).**

Results: Results showed that polished samples presented a lower high profile mean ($p<0.001$); however, YZHTg presented less space between defects ($p=0.0002$). SFE showed that YZHT presented higher SFE than ZLS. Profilometry evidenced more homogeneity on polished surfaces. The interaction of finishing technique and microorganisms influenced the CFU ($p=0.00$). MTT assay demonstrated initial severe cytotoxic behavior for polished surfaces. SEM images showed homogeneous surfaces, except for glazed YZHT.

Conclusion: Glazed surfaces have a greater roughness and tend to accumulate more biofilm. Polished surfaces have higher SFE; however, they are temporarily cytotoxic.

INTRODUCTION

Ceramics have become an alternative material for the manufacture of dental prostheses due to their esthetics and long-term proven resistance. With these advantages, their restorative techniques have greatly developed over time.[1] Zirconia partially stabilized by yttrium (YTZP) does not present a glass phase; however, it contains a highly crystalline phase and low translucency,[2] which confers opacity to visible light and the need for veneering infrastructures with esthetic ceramics.[3] Monolithic materials appeared with the purpose of combining adequate translucency and excellent mechanical properties,[4,5] aiming to overcome failures due to chipping of the veneering ceramic, decreasing clinical time and restoration costs.[6]

Recently, new formulations of zirconia reinforced lithium silicate (ZLS) ceramic materials have been introduced in the market (Celtra Duo, Dentsply, Konstanz, Baden-Württemberg, Germany; Suprinity, Vita Zahnfabrik), joining the existing group of ceramics and expanding the possibility of ceramic use in different clinical situations. According to the manufacturer, Vita Suprinity contains 56% to 64% SiO_2, 15% to 21% LiO_2, 1% to 4% K_2O, 3% to 8% P_2O_5, 1% to 4% Al_2O_3, 0% to 4% CeO_2, and 0% to 6% pigments, in addition to 10% zirconia, thereby presenting superior properties to lithium disilicate (LD), such as fracture toughness (2.31±0.17 MPa $m^{0.5}$), flexural strength (443.63±38.90 MPa), and elastic modulus (70.44±1.97 GPa). As disadvantages, ZLS proved to be harder (6.53±0.49 GPa) and more friable (2.84±0.26 $\mu m^{-1/2}$) than LD.[7] When used as an infrastructure, it is possible to leave zirconia exposed to oral medium due to its biocompatibility and lower tendency to accumulate oral biofilm.[8,9] However, the outcome of exposure of monolithic crowns of high translucency zirconia partially stabilized by yttrium (YZHT) and ZLS to oral fluids is not fully elucidated.

Bacterial adhesion to a substrate and the initial biofilm composition is related to topography,[10-12] surface hydrophobicity,[13,14] and communication between existing microorganisms.[15] If the surface is hydrophilic, a water pellicle will be present, making direct contact between the hydrophobic microorganism and the substrate difficult. *Streptococcus* is one of the first colonizers of initial supragingival biofilm in the first 8 hours[16] and is present in greater quantity in the oral biofilm.[17] Due to technological and clinical advances, ceramics tend to promote excellent marginal adaptation, finishing, and polishing. Zirconia has a less homogeneous surface compared with other materials because of pores resulting from the sintering process[18] or defects caused by polishing; these defects are due to the larger grains found in zirconia, and those grains susceptibility to being exposed during polishing.[19] Bacteria present in the oral cavity naturally tend to adhere to ceramic materials or to the interface between tooth and restoration,[11] the cervical third of the proximal surface, and along the gingival margin, where they are protected from mechanical action.[20] Oral biofilm is one of the best described microbial systems,[21] so it is well known that there is a mechanism for bacterial adherence and biofilm formation. On solid surfaces such as enamel, the ability to aggregate, the order of appearance of the microorganisms[17] and the environment[21] are important factors in oral biofilm formation. There is no consensus about the finishing technique that promotes the best surface smoothness in ceramics.[22,23] In the same way, to the knowledge of the authors, no studies have evaluated the interaction between different finishing techniques on the surface properties of these new materials, the formation of oral biofilm, or human gingival fibroblast (FMM-1)

viability when in contact with these monolithic ceramics.

Therefore, this study aimed to evaluate the influence of two finishing techniques (polishing or glazing) on the surface properties of two monolithic ceramics, as well as initial heterotypic biofilm formation *in vitro* and human gingival fibroblast (FMM-1) viability in contact with these ceramics. The null hypothesis was that surfaces resulting from polishing or glazing do not influence bacterial adhesion or FMM-1 cell viability.

METHODS AND MATERIALS

Vita YZ HT (YZHT; Vita Zahnfabrik, Bad, Säckingen, Germany; batch number 48980) and Vita Suprinity (ZLS; Vita Zahnfabrik; batch number 49142) were cut with a diamond disk in a cutting machine (Isomet 1000, Precision Sectioning Saw, Buehler, Lake Bluff, IL, USA) under constant cooling. In total, 92 specimens of each material were obtained, which were then sanded to standardize their dimensions in an automatic polisher (EcoMet/AutoMet250, Buehler) using sandpapers of decreasing grit up to #1200 (30 seconds per grit), and under water cooling. After cleaning in an ultrasonic bath with isopropyl alcohol (5 minutes), the specimens were sintered in their specific ovens. The final dimensions for both materials were 4.5 × 4.5 × 1.5 mm. Half of the blocks received a thin layer of Vita Akzent Spray HT glaze (Vita Zahnfabrik; batch number E33820) on its working side. The other half was submitted to the two step polishing protocol suggested by the manufacturer for both monolithic ceramics (VITA Suprinity Polishing Set clinical; batch number E6510). The specimens were randomly divided into four groups according to material (YZHT or ZLS) and finishing technique (g = glazing; or p = polishing), namely: YZHTg, YZHTp, ZLSg, ZLSp (Figure 1).

Surface Roughness (SR)

Twenty specimens from each group were analyzed by a contact rugosimeter (SJ 400, Mitutoyo, Tokyo, Japan) and a digital optical profiler (Wyko, ModelNT 1100, Veeco Instruments Inc, Tucson, AZ, USA). For roughness, five measurements were performed for each specimen in 5 random different areas with a read length of 3 mm and speed of 0.2 mm/s. The analysis was performed following ISO 4287-1997 standards, with Gaussian Filter and cut-off wavelength value of 0.8 mm. Average values were calculated for each sample, and the mean Ra and RSm (μm) values were obtained. For profilometry, specific software (WykoVision 32, Veeco Instruments Inc) was used for three-dimensional parameter measurements at 20× magnification in an area of 301.3 × 229.2 μm of two samples from each group.

Surface Free Energy (SFE)

Five samples (14×12×1.5 mm) from each group (obtained in the same way as described before) were used in conducting the SFE analysis by goniometer. An optical tensiometer (TL 1000, Theta Lite, OneAttension, Biolin Scientific, Lichfield, UK) was used to measure the mean contact angle (CAm) on five different areas by the sessile drop technique. Two liquids with different surface tensions were used: distilled water and diiodomethane,[24] at room temperature. In this technique, a graduated syringe (Gastight Syringes #1001 – 1ml, Hamilton, Reno, NV, USA) with a hydrophobic needle deposits a drop, and after 5 seconds the CAm is calculated with 60 images per second over 10 seconds. The SFE (mJ/m^2) was calculated according to the method proposed by Owens and Wend[25] using the harmonic average formula (equations 1 and 2) and information relating to the liquids[24]. The CAm was replaced to isolate the dispersive and polar constants of each solid. The sum of these constants correspond to the SFE (W or γ).

$$W_{12A} = \gamma_{1A}(1 + \cos\theta_A) = \frac{4\gamma_{1A}^d\gamma_2^d}{\gamma_{1A}^d + \gamma_2^d} + \frac{4\gamma_{1A}^P\gamma_2^P}{\gamma_{1A}^P + \gamma_2^P} \quad (1)$$

$$W_{12B} = \gamma_{1B}(1 + \cos\theta_B) = \frac{4\gamma_{1B}^d\gamma_2^d}{\gamma_{1B}^d + \gamma_2^d} + \frac{4\gamma_{1B}^P\gamma_2^P}{\gamma_{1B}^P + \gamma_2^P} \quad (2)$$

Where: γ corresponds to the SFE of the liquid, $\cos\theta$ = cosine of the liquid CAm, respectively for diiodomethane (1A) and water (1B). γ^d correponds to the dispersive energy and γ^p, to the polar energy. $\gamma_2{}^d$ and $\gamma_2{}^p$ correspond to the solid energies.

Colony Forming Units (CFUs)

Standard suspensions of *Streptococcus* (UA 159), *Streptococcus sanguinis* (ATCC 35688), and *Candida albicans* (ATCC 18804) were prepared containing 10^6 cells/mL (24 hours, 37°C). *Streptococcus* was cultured under microaerophilic conditions on brain heart infusion (BHI) broth supplemented with 15% glucose, and *C albicans* was cultured for 18 hours at 37°C in yeast nitrogen base broth (YNB; Difco, Detroit, MI, USA) supplemented with 100 mM of glucose. After incubation, the growth was suspended in a sterile physiological solution (0.9% sodium chloride [NaCl]),

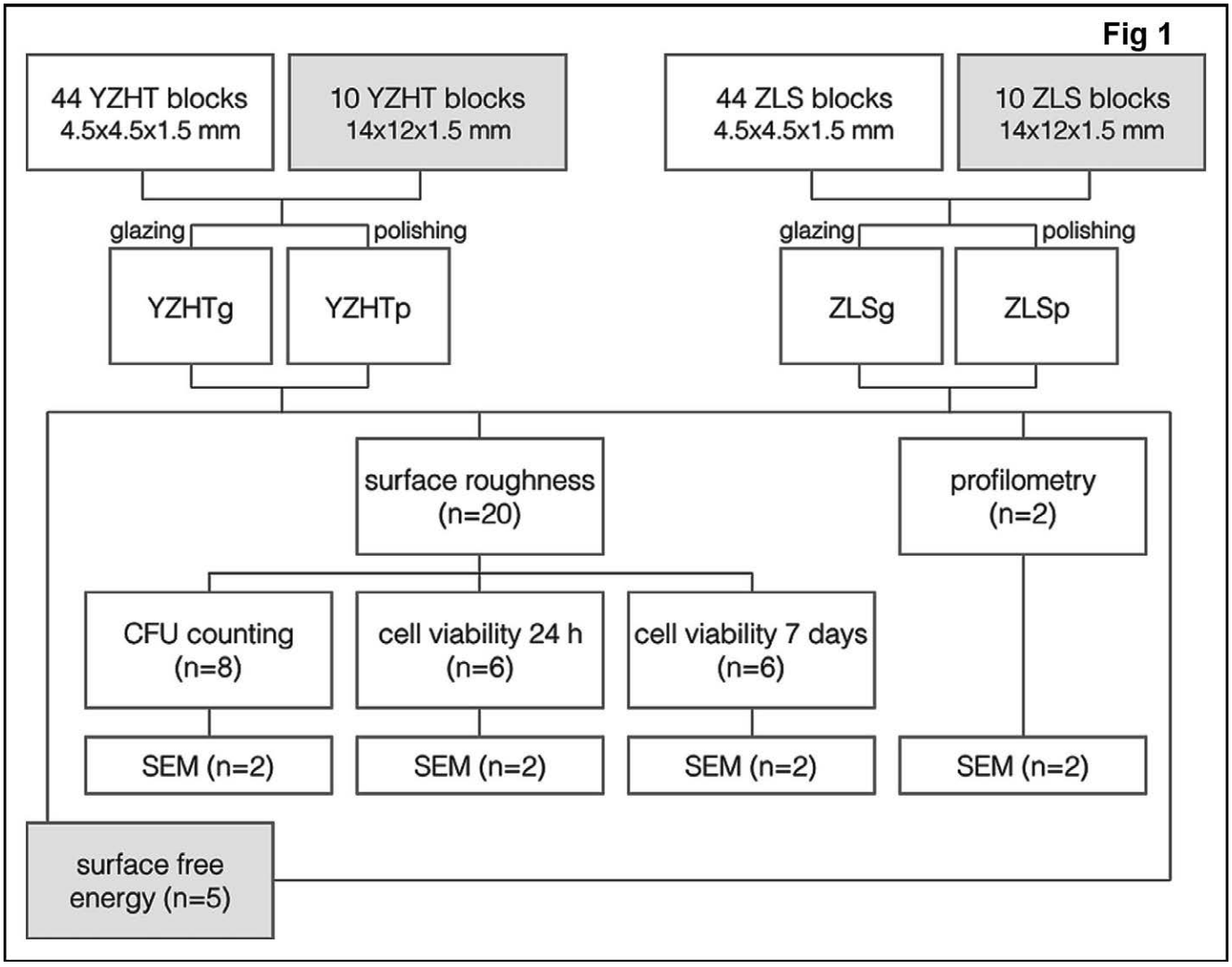

Figure 1. *Flow chart of tested samples. After nondestructive analysis (surface roughness and profilometry), samples were reused. In the surface free energy analysis, 14 × 12 × 1.5 mm blocks were used (grey boxes).*

and the number of cells in suspension was counted in a spectrophotometer (B582 – Micronal, São Paulo, Brazil). The parameters of optical density and wavelength used were 0.620 and 398 nm for *S mutans*, 0.560 and 398 nm for *S sanguinis* and 0.284 and 530 nm for *C albicans,* respectively. These parameters were previously established using a standard curve for CFU vs absorbance. Eight specimens of each group were sterilized in laminar flux (15 min each side), and each one was then distributed on a sterile 96-well polystyrene tissue culture plate. Next, each plate was contaminated with the association of all microorganisms (16.5 μL of each microorganism suspension, 70 μL of YNB and 30 μL of BHI supplemented with 1% of glucose). After this period, samples were removed and washed with sterile 0.9% NaCl in order to remove weakly bonded cells. The samples were individually placed in tubes with 10 mL of sterile 0.9% NaCl and sonicated (Sonoplus HD 2200, 30 W, Bandelin Eletronic, Berlin, Germany) for 30 seconds to disperse the biofilms. The suspension obtained was diluted 10^{-3} times for *C albicans* and 10^{-5} times for *Streptococcus*. Aliquots of 0.1 mL were seeded in duplicate onto petri plates with selective medium for each microorganism, as follows: Mitis Salivarius agar (Difco) for *S sanguinis*, Mitis Salivarius agar (Difco) with 0.2 UI/mL of bacitracin (União Química, Sao Paulo, Brazil) and sucrose (MSBS) for *S mutans*, and Sabouraud dextrose agar with 50 mg/L of clorafenicol (União Química) for *C albicans*. The plates were incubated for 16 hours at 37°C in a CO_2 chamber. Then, the plates with 30 to 300 typical

Table 1: *Mean Values (μm) ± SD, 95% CV, Median (μm), Kruskal-Wallis Analysis Results (p-value and Kruskal-Wallis Statistic), and Homogeneous Groups from the Dunn Test for Roughness Values in Ra and RSm Parameters*[a]

Material	Ra			RSm		
	Mean ± SD	95% CV	Median	Mean ± SD	95% CV	Median
YZHTg	2.37 ± 0.97	40.85%	2.45[A]	128.5 ± 51.92	40.49%	109.0[B]
YZHTp	0.58 ± 0.23	39.82%	0.55[C]	103.2 ± 101.60	98.48%	215.0[A]
ZLSg	0.96 ± 0.36	37.21%	1.00[B]	258.1 ± 112.40	43.55%	244.6[A]
ZLSp	0.33 ± 0.18	55.03%	0.31[C]	73.40 ± 49.40	67.30%	240.4[A]
Kruskal Wallis	55.65			19.37		
p value	<0.001			0.0002		

Abbreviations: CV, coefficient of variation; SD, standard deviation; YZHTg, zirconia partially stabilized by yttrium with glazing; YZHTp, zirconia partially stabilized by yttrium with polishing; ZLSg, zirconia reinforced lithium silicate with glazing; ZLSp, zirconia reinforced lithium silicate with polishing.
[a] *Groups with similar letters do not present statistical difference.*

colonies were counted and mean values of CFU/mL were obtained.

FMM-1 Cell Viability Assay

Cell viability was determined by measuring mitochondrial function based on its capability to reduce MTT (3-[4.5-dimethyl-thiazol-2-yl-] -2.5-diphenyl tetrazolium bromide) into a colored formazan product. Cell viability was quantified by dissolving MTT (Sigma, St Louis, MO, USA) in 0.1 N NaOH (6.25 v/v%) in DMSO (dimethyl sulfoxide), and cell survival was expressed as a percentage in relation to control group (=100%) consisting of plates without ceramic material. The standard curve was evaluated to convert optical density values to the number of viable cells, using cell densities of 2×10^4 cells/well. Twelve samples from each group were used to evaluate cell viability after 24 hours and 7 days. The medium was replaced every 48 hours over the 7 days. The cell monolayer at the bottom of the wells was washed with 500 mL of PBS. Then, 500 μl of MTT solution (0.5 mg/mL PBS) was added to each well. The plates were incubated (1 hour at 37°C) in the absence of light and supernatants were discarded. After the wells were washed with 500 μl PBS, the plates were incubated in DMSO solution (10 min, 37°C) and shaken on an orbital table (10 minutes). Finally, 100 μL of supernatant from each well was placed in triplicate in a 96-well plate and read at 570 nm (EL808IU, Biotek, Winooski, VT, USA).

Scanning Electron Microscopy (SEM)

Polished and glazed surfaces free of microorganisms, as well as contaminated specimens with initial biofilm and with FMM-1 cells, were observed and photographed by SEM (Inspect S 50 – FEI Company, Brno, Czech Republic), operating at 15 kV. Samples with cells or microorganisms were fixed for 1 hour in 2.5% glutaraldehyde, dehydrated in several ethanol washes (10%, 25%, 50%, 75%, and 90% for 20 minutes and 100% for 1 hour) and dried overnight in a bacteriologic incubator at 37°C. All samples received a gold coat in a low-pressure atmosphere using an ion sputter coater (Polaron SC 7620 Sputter Coater, Quorum Technologies, Newhaven, UK).

Statistical Analysis

Once the normality of the data using Kolmogorov-Smirnov test was confirmed, SFE results (mN/m) were statistically analyzed by two-way analysis of variance (ANOVA), CFU data (in log10) and MTT (in %) were analyzed by three-way ANOVA, all with $\alpha = 0.05$, using Minitab software (Minitab 17 for Windows, 2004, State College, Pennsylvania, USA). Tukey test was used to detect differences (α=0.05). Because the distribution was not normal, roughness data (μm) were submitted to Kruskal-Wallis, Dunn, and Mann-Whitney tests (all, α=0.05). Images obtained by profilometry and SEM were qualitatively analyzed.

Results

SR

Table 1 presents the descriptive statistical analysis for roughness data. The Kruskal-Wallis test showed that both roughness parameters were influenced by finishing techniques and material ($p<0.05$; Table 1). The YZHTg group showed higher (Ra) and less spaced (RSm) grooves on average compared with the others. The Mann Whitney test indicated that finishing technique only influenced the Ra parameter, while material influenced both parameters (Table 2). Three-dimensional profilometry images (Figure 2) emphasize the statistical differences observed by SR between glazed and polished ceramics. Glazed surfaces are rougher than polished

Table 2: *Descriptive Statistics (Median, in μm), Results of Mann-Whitney Analysis (p-Value and W statistic) of Roughness Values for Material and Finishing Technique Isolated Factors*[a]

Parameters	Polishing	Glazing	Surface		YZHT	ZLS	Material	
	Median	Median	*p* value	W	Median	Median	*p* value	W
Ra	0.40	1.30	**0.0000**	937	1.00	0.50	**0.004**	438
RSm	215.0	167.40	0.52	1.686.0	186.3	241.0	**0.027**	493.0

Abbreviations: YZHT, zirconia partially stabilized by yttrium; ZLS, zirconia reinforced lithium silicate.
[a] *Bold p values were statistically significant.*

surfaces. The YZHTg surface was more heterogeneous than ZLSg. For polished surfaces, ZLS was more homogeneous but the presence of polishing grooves was noted on YZHT ceramic.

SFE

CAm data and the resulting SFE are described in Table 3. Both glazed and polished ceramics presented a predominantly hydrophilic behavior. The interaction between material and finishing technique influenced the SFE (p=0.001), and Tukey test showed that YZHT (glazed or polished) presented higher SFE than ZLS, while ZLSp and ZLSg were similar to each other.

CFU/mL

Three-way ANOVA showed a statistical difference for the interaction between finishing technique and microorganism. The mean values of CFU transformed into log 10 base for all experimental groups according to the interaction are shown in Table 4. *C albicans* formed fewer CFUs per milliliter on ceramics, with statistically higher adhesion on glazed surfaces.

Cell Viability Assay

The MTT assay results indicated that FMM-1 cells in early contact with the ceramics (24 hours) or with longer exposure (7 days) did not cause enough damage to characterize them as cytotoxic materials, except for the polished groups, which presented cellular viability lower than 50% after 24 hours, thus characterizing them with severe cytotoxicity, according to the International Organization of Standardization 10993-5.[26] Student t-test showed difference in cell viability between both evaluated periods (24 hours and 7 days) and control group considered 100%, (p<0.05). Three-way ANOVA showed that only period of contact influenced cell growth on the ceramics (p=0.00). Tukey test identified that ceramics in contact with the cells over 7 days had a higher number of viable cells compared with the 24-hour period, regardless of material or surface treatment. The YZHTg group was the only one that presented a decrease in mean cell viability (5.1%) between 24 hours and 7 days, whereas the others presented variable increases (Figure 3).

SEM

The surface micrographs of sterile (1000×, Figure 2, middle column) and contaminated materials (3000×, Figure 2, right column) allowed for observing different surface patterns. The glaze layer on YZHT ceramic was less homogeneous than on ZLS. The

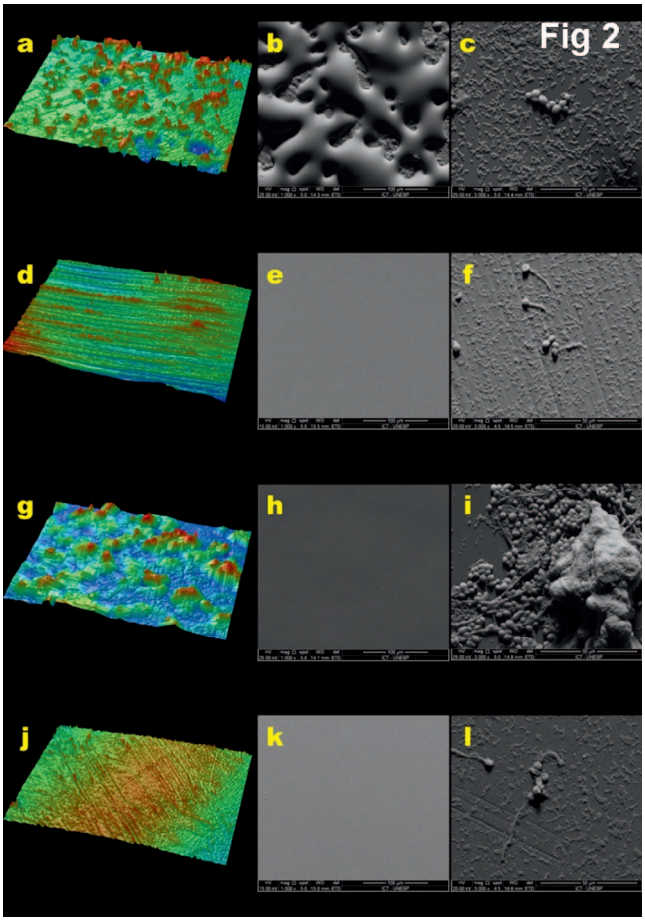

Figure 2. *Images from three-dimensional profilometry, SEM of the surfaces and SEM of contaminated surfaces, respectively, for the groups (a-c) YZHTg, (d-f) YZHTp, (g-i) ZLSg, and,(j-l) ZLSp.*

Table 3: *Mean Contact Angle ± SD for Water and Diiodomethane, Dispersive (γ_d, in mN/m) and Polar (γ_p, in mN/m) components and Respective Surface Free Energy (γ_T, in mN/m) of the Ceramics With Evaluated Finishing Techniques*[a]

Material	Mean Contact Angle		Components		
	Water Mean ± SD (°)	Diiodomethane Mean ± SD (°)	γ_d (mN/m)	γ_p (mN/m)	γ_T (mN/m)
YZHTg	51 ± 11	47 ± 2	37.5	38.0	75.5[B]
YZHTp	86 ± 10	54 ± 5	33.2	57.4	90.6[C]
ZLSg	32 ± 24	49 ± 7	37.5	33.0	71.5[A]
ZLSp	19 ±4	53 ± 5	34.0	39.0	73.0[AB]

Abbreviations: SD, standard deviation; YZHTg, zirconia partially stabilized by yttrium with glazing; YZHTp, zirconia partially stabilized by yttrium with polishing; ZLSg, zirconia reinforced lithium silicate with glazing; ZLSp, zirconia reinforced lithium silicate with polishing.
[a] Identical upper case letters represent absence of statistical difference by Tukey test (α=0.05).

roughness pattern generated by the polishing technique was homogeneous and similar between materials. The presence of *Streptococcus* and *C albicans* was observed on contaminated samples. An increase in the number of FMM-1 cells adhered to the materials' surface submitted to the MTT assay was observed in relation to time (Figure 4), independent of the surface morphology.

DISCUSSION

This study aimed to evaluate the interaction between surface properties and biofilm formation for the viability of human gingival fibroblasts. The results rejected the null hypothesis. The clinical long-term success of dental ceramics depends on their physical properties, manufacturing process, laboratory manufacturing technique, and clinical procedures. The composition of the material, as well as its surface structure, can influence the initial bacterial adhesion and compromise dental health.[10,12] Ceramics are attractive restorative materials due to their esthetic quality and biocompatibility; the smooth surfaces minimize oral biofilm accumulation.[13]

Table 4: *Mean Values ± SD in log10 of the Amount of CFU and Homogeneous Groups According to Tukey test for the Interaction Finishing technique*Microorganism* [a]

Finishing Technique *Microorganism	Mean ± SD (log10)
Glazed**mutans*	7.72 ± 0.13[A]
Glazed**sanguinis*	7.60 ± 0.19[A]
Polished**sanguinis*	6.60 ±0.31[B]
Polished**mutans*	6.55 ± 0.27[B]
Glazed**candida*	5.24 ± 0.23[C]
Polished**candida*	0.26 ± 1.52[D]

Abbreviations: CFU, colony forming unit; log10, log of base 10; SD, standard deviation.
[a] Identical upper case letters indicate absence of statistically significant difference.

For roughness analysis and qualitative assessments, high translucency zirconia (YZHT) presented a rougher profile than lithium silicate reinforced by zirconia (ZLS). Results from profilometry and SEM analyses corroborate YZHTg as having the highest absolute mean height of irregularities along the profile. Regardless of ceramic material, glaze application resulted in rougher surfaces. Another fact contributing to greater roughness in YZHTg may be the chemical union between glaze and zirconia, where the glaze has accumulated in islands. This accumulation causes unevenness between surface and glaze, resulting in higher Ra values for YZHT. The glaze layer on vitreous ceramics is distributed more evenly, increasing the spacing between peaks and valleys (higher RSm).

ZLSp was more hydrophilic regarding the mean contact angle (CAm) between water and ceramic. According to Shirtcliffe and others,[14] a surface with CAm to water between 0 and 180° is characterized as partially hydrophilic. A surface with a hydrophobic tendency may have this feature raised by increasing the roughness,[13,14] affecting its wettability and thus favoring bacterial retention.[13] This was observed for ZLS which presented high values of Ra and higher CAm to water when glazed. Therefore, it is important that clinicians have knowledge about the consequences of inadequate procedures that result in rougher surfaces,[27] which may be contaminated with impurities or modified by exposure to changes in temperature, which in turn may increase SFE.[28] According to Anusavice,[29] SFE is directly associated with adhesion. Thus, YZHTp may be suggested as the condition that results in a better adhesive property.

The presence of glaze on the surface does not prevent the formation of dental biofilm, as observed in a previous study[23] that compared *C albicans* adhesion on a porcelain surface without surface treatment, glazed and polished. The authors verified

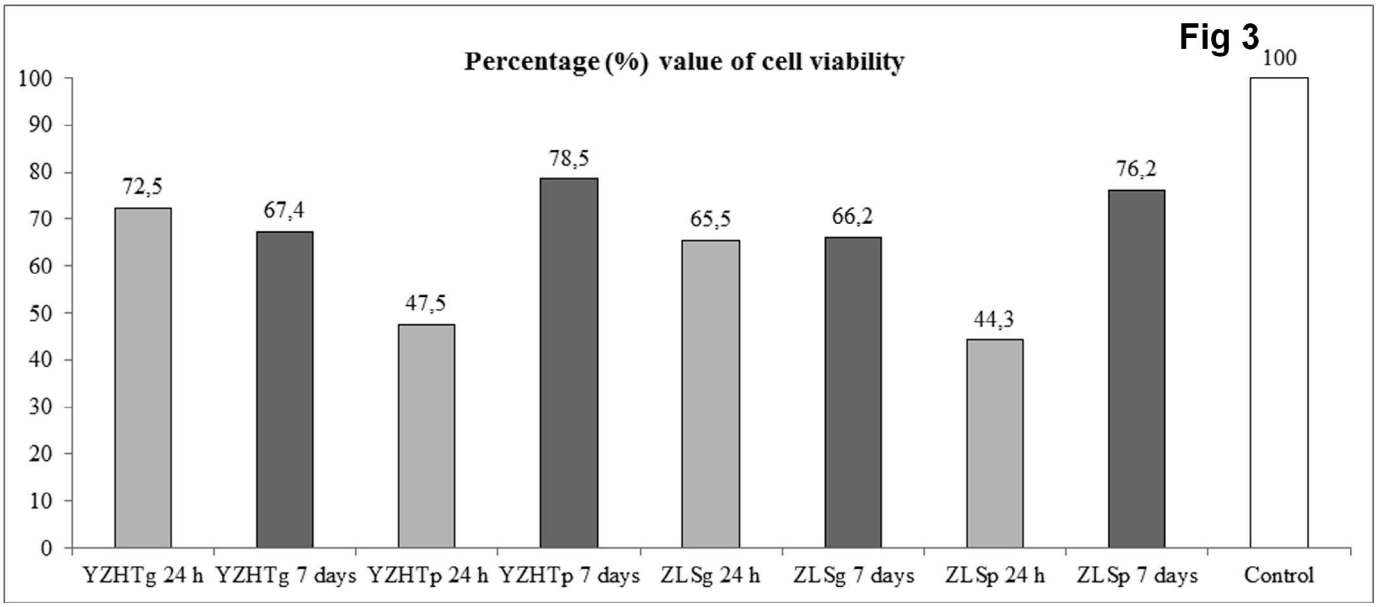

Figure 3. *Bar graph for percentage of viable cells through MTT assay at 24 hours and 7 days.*

that the glazed surface had a lower value of bacterial adhesion compared with the surface without superficial treatment, but there was no significant difference from the polished group.[23] In mature biofilm, the adherence of microorganisms occurs on other layers of microorganisms. Therefore, this study simulated *in vitro* environmental conditions for the formation of an initial biofilm in order to evaluate the interaction between microorganisms and the surface of the materials. Recent studies have validated different periods up to 24 hours for the formation of such biofilms, using two or more microorganisms.[30,31]

In this study, we examined the adherence of an initial colonizer (*S sanguinis*), a colonizer associated with the development of carious lesions (*S mutans*), and finally, a colonizer related to caries, periodontal diseases, and candidiasis (*C albicans*). The availability of studies evaluating bacterial adhesion to monolithic ceramics is scarce. The adhesion of *S mitis* and *Prevotella nigrescens* on Metoxit AG zirconia (High Tech Ceramics, Thayngen, Switzerland), for example, is lower than on titanium used in manufacturing dental implants (Goodfellow Cambridge Limited, Huntingdon, UK).[31] A previous study verified that glazed Lava zirconia (3M ESPE, St. Paul, MN, USA) presented greater roughness compared with the polished surface, and there was a tendency toward biofilm accumulation.[8] When microorganisms were compared to each other, a greater growth of *Streptococcus* was observed independent of the surface type. Also, a greater formation of colonies on glazed surfaces was observed in comparison to polished surfaces, corroborating a previous study.[8] This may be associated with the fact that *S sanguinis* facilitates the growth of other *Streptococcus* that grow in a similar way, which can be justified by their hydrophobic nature.[31,32] *C albicans* also presents a hydrophobic characteristic; however, its smaller growth may be associated with the fact that *Streptococcus* is a commensal microorganism,[21] where both species are associated with benefits for one of them without harming the other. In the ceramic structure, *C albicans* acts as a facilitator for the adherence of *S mutans*.[33] The low growth of *C albicans* on polished surfaces can be justified by its difficulty in adhering to very smooth surfaces compared with *S mutans*,[34] since the second produces a water-insoluble substance that facilitates the adherence of these microorganisms to a smooth substrate.[35] Different from rough surfaces, polished surfaces do not accumulate many nutrients. This dispute over scarce food, as well as the lack of space and negative effect of metabolites from bacteria, can also justify a competition between *C albicans* and *Streptococcus*. The interaction between *S mutans* and *C albicans* is given by mutualism, where microorganisms benefit, resulting in mutual dependence.[36] It is interesting to observe the small number of *C albicans* colonies because healthy tissue free of fungus in restorations where the dental preparation has contact with the gingival tissue is necessary. A lower number of *C albicans* colonies on smooth surfaces has also been reported for other materials

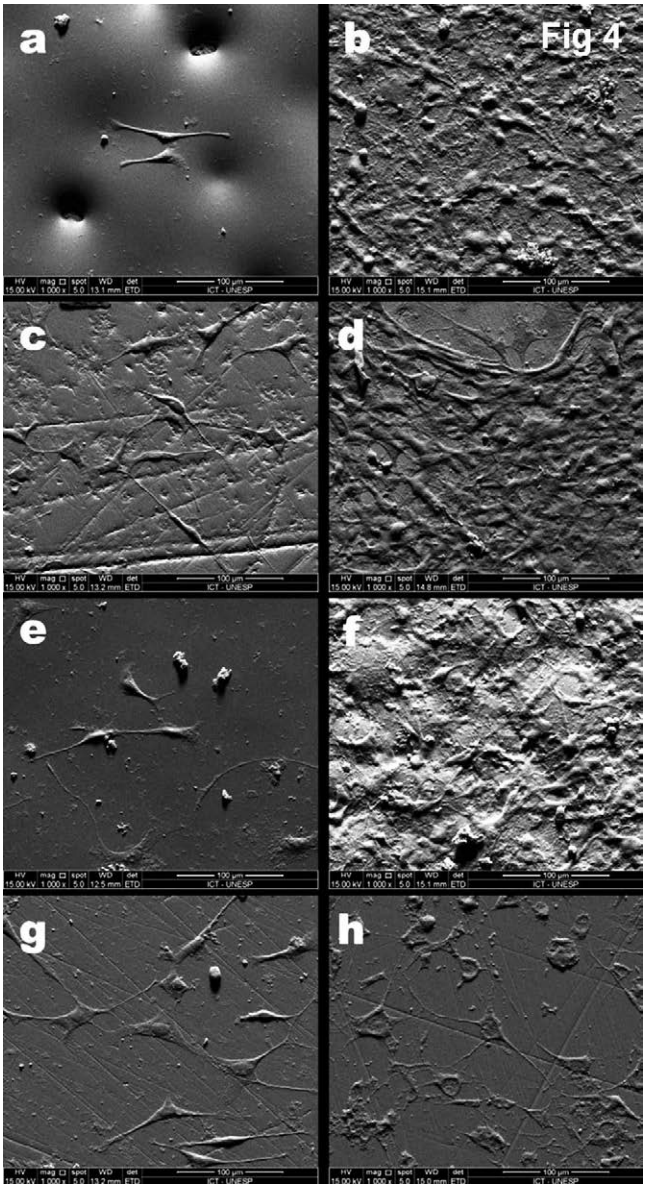

Figure 4. *SEM images of FMM-1 cells in contact with the ceramics YZHTg (a-b), YZHTp (c-d), ZLSg (e-f), and ZLSp (g-h) for the periods of 24 hours and 7 days, respectively. Magnification of 1000×.*

used in the oral cavity.[35] The results show that a higher number of microorganisms adhered to the roughened surfaces, presenting less SFE than polished surfaces. In this way, the roughness seems to be the main factor related to biofilm formation. Considering smooth surfaces, it is suggested that SFE may be the main factor associated with initial bacterial adherence.[31,34]

It is possible to verify the presence of *Streptococcus* and *C albicans* (Figure 2, right column) in the SEM images. The ceramics were colonized with a thin biofilm filled with cellular agglomerates of similar size and morphology, with an emphasis on the extensive colonization by *Streptococcus*, and it was not possible to distinguish *Streptococcus*. The amorphous substance is an important factor in the relationship of all studied microorganisms. In Figure 2 (left column), SEM images show this amorphous matrix involving the species, visibly larger on ZLSg. This matrix may be associated with the adhesion of *C albicans* to the biofilm. The presence of this fungus corroborates the assertion that this facilitates the adherence of *S mutans*, and may be associated with an increased risk of caries. The results show that both materials under both finishing techniques, can be considered moderately cytotoxic[26] to the growth of human gingival fibroblasts (FMM-1), since all groups presented cellular viability between 50% and 79%. The initial (24 hour) cytotoxicity of polished groups may be related to the release of some substance at this initial time, reducing its cytotoxic effect after 7 days. This initial cytotoxicity may occur if the cells do not present sufficient immediate defense to some remnant of the polishing procedure. Over time, the cells enhance their defense mechanisms and become capable of protecting themselves from the aggressor. Therefore, future studies evaluating which substances are released causing tissue damage are important.

CONCLUSION

ZLS resulted in lower mean roughness profile and more spaced defects regardless of surface finishing. Polished surfaces were less rough and presented higher SFE, but they also showed severe initial cytotoxicity when in contact with FMM-1 cells. However, they were inert in the long term.

Acknowledgements

The authors would like to thank the Foundation for Research Support of the State of São Paulo (FAPESP) for the scholarship, through the process numbers 2015/13322-1 and 2014/19357-9. The authors also would like to thank Vita Zahnfabrik and Wilcos from Brazil and CAPES (Coordination for Improvement of Higher Education Staff) for their support.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institute of Science and Technology, São Paulo State University, Brazil.

Conflict of Interest

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

(Accepted 5 April 2017)

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Effect of Bleaching Gel Viscosity on Tooth Whitening Efficacy and Pulp Chamber Penetration: An *In Vitro* Study

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Clinical Relevance

The viscosity of the whitening gel or delivery system did not influence efficacy but affected hydrogen peroxide penetration that may relate to increased tooth sensitivity with lower viscosity gels.

SUMMARY

Objectives: Whitening efficacy has been related to hydrogen peroxide (HP) diffusion into tooth structure. However, little information is available relating rheological properties to whitening efficacy. The purpose was to evaluate the whitening efficacy and HP penetration level of a 10% HP gel at three different viscosities and to compare them to a strip delivery system.

Methods and Materials: Extracted molars (n=120) were randomly assigned into five groups (n=24/ group): NC_MED (negative control; median): medium viscosity gel without HP; LOW: 10% HP gel (low viscosity experimental gel, Ultradent Products Inc); MED: 10% HP gel (medium viscosity experimental gel, Ultradent); HIGH: 10% HP gel (high viscosity gel, Ultradent); and CWS: Crest 3D Whitestrips 1-Hour Express (Procter & Gamble). All teeth were subjected to five 60-minute whitening sessions. Instrumental color measurements were performed at baseline (T_0), and 1-day after each application (T_1-T_5), and 1-month after whitening (T_6). HP penetration was estimated with leucocrystal violet and horseradish peroxidase. A Kruskal-Wallis test and *post hoc* Bonferroni test were performed to assess the difference in tooth color change and HP penetration among the groups (α=0.05).

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DOI: 10.2341/17-099-L

Results: Hydrogen peroxide penetration levels and overall color changes at T_6 were 0.24 μg/mL / 2.80; 0.48 μg/mL / 8.48; 0.44 μg/mL / 7.72; 0.35 μg/mL / 8.49; 0.36 μg/mL / 7.30 for groups NC, LOW, MED, HIGH, and CWS, respectively. There was a significant difference for HP penetration, while there was no significant difference among the four experimental groups for tooth color change.

Conclusion: Rheological properties should be considered when developing new whitening formulations.

INTRODUCTION

Demand for healthy and beautiful teeth has been building and growing for more than a generation, as the general public envisions and desires a "Hollywood" smile. According to the American Academy of Cosmetic Dentistry, the teeth whitening industry surpassed an annual revenue of $11 billion at the beginning of 2015, with $1.4 billion spent on tooth whitening products.[1] Additionally, statistics show that over 90% of adults believe that a healthy, white smile makes you look more appealing, while over 70% believe that a person's smile can be a critical factor in career advancement.[1] This perception may explain why so many people are willing to invest in teeth whitening. The wide range of whitening modalities available also reflects the high demand: professionally applied in-office whitening; professionally dispensed, patient-applied home whitening; over-the-counter whitening products; and do-it-yourself whitening.[2]

Even though its use is ubiquitous in dentistry around the world and extensively studied, there is a need for further development of innovative and user-friendly whitening systems that are efficient and also safe to use. Studies on diffusion have shown that hydrogen peroxide (HP), which is the primary active agent in most tooth whitening materials, readily penetrates into the pulp cavity in 5 to 15 minutes when applied to the external surface of the tooth.[3,4] The penetration increases with the use of higher peroxide concentration, heat, light, and younger age of the tooth.[4-7] Correlations of HP penetration levels with whitening efficacy did not show any positive relationship.[8]

Most whitening materials are gel-based systems and are manufactured in different viscosities. However, little information is available regarding the effect of rheological properties and delivery systems on tooth whitening effectiveness and HP penetration. Therefore, the purpose of this study was to evaluate the whitening efficacy and hydrogen peroxide penetration level of a 10% HP gel at three different viscosities (low, medium, and high) and also compare them with a strip delivery system. The first null hypothesis tested was that there would be no difference in tooth whitening efficacy regarding overall color change (ΔE*), lightness change (ΔL*), and chroma change in the yellow-blue axis (Δb*) among the different experimental viscosities and delivery systems tested. The second null hypothesis was there would be no difference in HP penetration levels into the pulp cavity among the groups.

METHODS AND MATERIALS

Sample Selection and Preparation

Extracted human molar teeth (n=120) were collected and stored in 0.1% thymol solution (Sigma-Aldrich, St Louis, MO, USA) at 4°C. All teeth were cleaned and checked for the presence of anomalies, caries, existing restorations, crack lines, and severe attrition. The roots were trimmed 2 mm apical to the cementoenamel junction with a sectioning machine (TechCut 4, Allied High Tech Products Inc, Compton, CA, USA). The pulp cavity was enlarged and prepared with tapered diamond burs (NeoDiamond, Microcopy, Kennesaw, GA, USA) toward the lingual with the purpose of establishing an intact labial tooth structure of 2-mm thickness that could contain 50 μL of acetate buffer. The occlusal pits and fissures were sealed with flowable resin (Permaflo, Ultradent Products Inc, Jordan, UT, USA) to prevent any leakage of the buffer out of the cavity. A circular adhesive label 6 mm in diameter was adhered at the center of the labial surface to establish a standardized color reading and whitening area. The remaining tooth was painted with gray nail varnish (Sally Hansen, New York, NY, USA), and then the adhesive label was removed after drying, leaving a standard-sized unpainted area of enamel for whitening application.

Application Protocol by Group

Specimens were randomized into five groups of 24 specimens each, as follows: Group NC_MED (negative control; median): MED viscosity gel without HP (experimental gel, Ultradent) acting as the negative control; Group LOW: 10% HP gel of low viscosity (experimental gel, Ultradent); Group MED: 10% HP gel of medium viscosity (experimental gel, Ultradent), Group HIGH: 10% HP gel of high viscosity (Opalescence GO in syringe delivery, Ultradent), and Group CWS: 10% HP in a strip delivery system

Table 1: *Summary of Whitening Agents Used by Group*

Group	Product	HP Concentration	Viscosity	Lot No
NC_MED	Experimental placebo gel	N/A	Medium	RH1016B
LOW	Experimental bleaching gel	≅ 10%	Low	RH0916A
MED	Experimental bleaching gel	≅ 10%	Medium	GH0916A
HIGH	Opalescence GO 10% HP gel	≅ 10%	High	TQAGL
CWS	Crest Whitestrips 1hr Express	≅ 10%	N/A	6193652600

(Crest 3D Whitestrips 1-Hour Express, Procter & Gamble, Cincinnati, OH, USA). Table 1 summarizes the whitening agents used by group.

A jig was fabricated for each specimen by gently placing the lingual surface of each tooth into an unset increment of polyvinyl siloxane impression material (Aquasil Ultra Heavy, Dentsply Caulk, Milford, DE, USA) at a 30° angle from the base. Whitening material (0.2 mL) was applied on an unpainted enamel surface and covered with a linear low-density polyethylene wrap (Saran Premium Wrap, SC Johnson & Son Inc, Racine, WI, USA) to simulate the placement of a custom fabricated tray (Figure 1). The teeth were kept at room temperature (25°C) during the treatment procedure. All teeth were subjected to a 60-min whitening session for 5 consecutive days and stored in artificial saliva at 4°C throughout the study.[9]

Color Measurement

A contact-type intraoral spectrophotometer (Vita Easyshade Compact Advance, Vita Zahnfabrik, Bad Säckingen, Germany) with a 5-mm diameter probe was used for instrumental color measurements. The Easyshade was calibrated and placed perpendicular and flush to the exposed tooth surface according to the manufacturer's instructions. Measurements were performed seven times each at baseline (T_0), 1 day after each application (T_1 to T_5), and 1 month after whitening (T_6). To standardize the environment, a color-controlled light box (MM 4e GTI Mini Matcher, GTI Graphic Technology Inc, Newburgh, NY, USA) at CIE D_{65}, a color temperature of 6500K, and light intensity of ≈1200 lux was used. Color difference was calculated as ΔE^*_{ab} from the following equation of the Commission Internationale de l'Eclairage:[10]

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

Spectrophotometric Assay of HP

HP penetration was measured after the first whitening session and estimated with leucocrystal violet and horseradish peroxidase. Acetate buffer (40 μL) retrieved from the pulp cavity was mixed with 1 mL leucocrystal violet solution (0.5 mg/mL), 0.5 mL of horseradish peroxidase solution (1 mg/mL), and 1 mL of acetate buffer.[11] The final color intensity was measured in an absorbance spectrophotometer (Benchmark, Bio-Rad, Hercules, CA, USA) at a wavelength of 600 nm. A standard calibration curve with known amounts of HP was used to determine the amount in microgram equivalents in the samples.

Measurement of Viscosity

Sample viscosities were measured as a function of shear rate using a stress-controlled rheometer (MCR

Figure 1. *Flow diagram of experimental protocol.*

Table 2: *Color Change (ΔL*, Δb*, and ΔE*) Over Time by Group (Mean±SD)*

Color Change	NC_Med	Low	Med	High	Cws	*P*-value*
ΔL_T1	−0.3±1.4^{Aa}	1.3±2.6^{AC}	1.7±1.5^{AC}	2.0±2.1^{BC}	1.2±3.3^{AC}	<0.001
Δb_T1	0.3±1.3^{A}	−0.6±2.1^{A}	−0.1±2.1^{A}	−0.5±2.1^{A}	0.0±1.9^{A}	0.247
ΔE_T1	1.8±0.9^{A}	3.0±2.2^{A}	2.8±1.4^{A}	3.3±1.71^{A}	3.1±2.5^{A}	0.03
ΔL_T2	1.2±1.7^{A}	3.2±2.6^{A}	2.5±1.7^{A}	2.3±2.0^{A}	1.9±3.4^{A}	0.064
Δb_T2	0.6±1.8^{A}	−1.8±2.3^{B}	−1.5±2.1^{B}	−1.9±1.8^{B}	−1.4±2.1^{B}	<0.001
ΔE_T2	2.5±1.3^{A}	4.4±2.6^{BC}	3.8±1.4^{AC}	3.8±1.6^{AC}	4.1±2.3^{AC}	0.006
ΔL_T3	0.3±1.9^{A}	2.8±2.6^{BC}	1.3±2.9^{AC}	3.2±2.0^{BC}	1.5±3.4^{AC}	<0.001
Δb_T3	−0.9±2.1^{A}	4.0±2.8^{B}	−4.7±2.9^{B}	−4.0±2.4^{B}	−2.9±2.5^{AB}	<0.001
ΔE_T3	2.6±1.6^{A}	5.6±3.1^{B}	5.7±3.2^{B}	5.9±1.8^{B}	5.0±2.3^{B}	<0.001
ΔL_T4	1.5±1.5^{A}	4.5±2.7^{BC}	3.6±2.5^{AC}	4.6±2.1^{BC}	3.2±3.3^{AC}	<0.001
Δb_T4	0.8±2.1^{A}	−3.3±2.6^{B}	−3.8±2.8^{B}	−3.8±2.3^{B}	−3.8±2.0^{B}	<0.001
ΔE_T4	2.7±1.5^{A}	6.1±3.1^{B}	6.0±2.6^{B}	6.7±1.6^{B}	6.0±1.7^{B}	<0.001
ΔL_T5	1.6±1.5^{A}	4.0±2.8^{BC}	3.8±1.6^{BC}	4.1±2.2^{BC}	2.9±3.0^{AC}	<0.001
Δb_T5	0.7±1.6^{A}	−4.6±2.3^{B}	−3.6±2.4^{B}	−4.2±2.2^{B}	−3.8±1.7^{B}	<0.001
ΔE_T5	2.5±1.3^{A}	6.5±3.2^{B}	5.7±2.1^{B}	6.5±2.0^{B}	5.7±1.6^{B}	<0.001
ΔL_T6	1.3±1.8^{A}	5.2±3.2^{BC}	4.0±2.3^{BC}	5.0±2.7^{BC}	3.4±3.2^{AC}	<0.001
Δb_T6	−0.1±2.0^{A}	−5.7±2.8^{B}	−5.8±3.1^{B}	−5.5±3.0^{B}	5.2±2.4^{B}	<0.001
ΔE_T6	2.8±1.4^{A}	8.5±3.2^{B}	7.7±2.9^{B}	8.5±1.8^{B}	7.3±2.2^{B}	<0.001

* *Kruskal-Wallis test.*
[a] *Same letters within a given row indicate no significant differences, based on* post hoc *test adjusted for multiple comparisons.*

301, Anton-Paar GmbH, Graz, Austria) and cone-and-plate geometry with a sandblasted 2°, 25-mm diameter cone at a gap height of 0.052 mm. The rheometer was equipped with an integrated Peltier heating/cooling system and a Peltier hood, to ensure a uniform temperature throughout the sample and minimize evaporation. All tests were performed at a constant temperature of 37°C. Four sets of measurements were conducted on each sample to assess reproducibility, with the data showing an average standard deviation of less than 5%. Flow ramp-up experiments were performed, and sample viscosities were recorded at 37 logarithmically spaced shear rates in the range $\dot{\lambda}$ = 0.1– 400 L/s. The results of the four independent experiments on each sample were averaged to report the viscosity at each shear rate.

Data Analysis

Descriptive statistics were conducted to profile all variables in the study. The Kruskal-Wallis, followed by the *post hoc* Bonferroni test, was performed to determine whether differences in color parameters and change in color parameters among the five groups at each post-whitening time point were statistically different. The change in each color parameter is defined as the difference between baseline and each post-whitening time point. The above-mentioned tests were also performed to assess the difference in HP penetration levels among the groups. Based on the power analysis, a sample size of 22 for each group was determined to have 80% power, with an alpha of 0.05 and effect size of ΔE* = 2.7. IBM SPSS Statistics Version 24 (IBM Corp, Armonk, NY, USA) was used for data analysis at a significance level of 0.05.

RESULTS

Tooth Color

There were no significant differences for baseline color parameters L_0*, a_0*, or b_0* (p=0.687, 0.980, and 0.868, respectively). Color change (ΔL*, Δb*, ΔE*) over time by group is summarized in Table 2. All groups except for NC_MED showed an increase in lightness and decrease in chroma in the yellow-blue axis over time (Figures 2 and 3). ΔL*, Δb*, and ΔE*were not significantly different among the four experimental groups from T_1 to T_6. All groups showed a significant separation from the negative control group at T_3, which persisted until the 1-month follow-up evaluation. Overall color changes at T_6 were 2.80, 8.48, 7.72, 8.49, and 7.30 for groups NC_MED, LOW, MED, HIGH, and CWS, respectively. Figure 4 illustrates the overall color change by group over time.

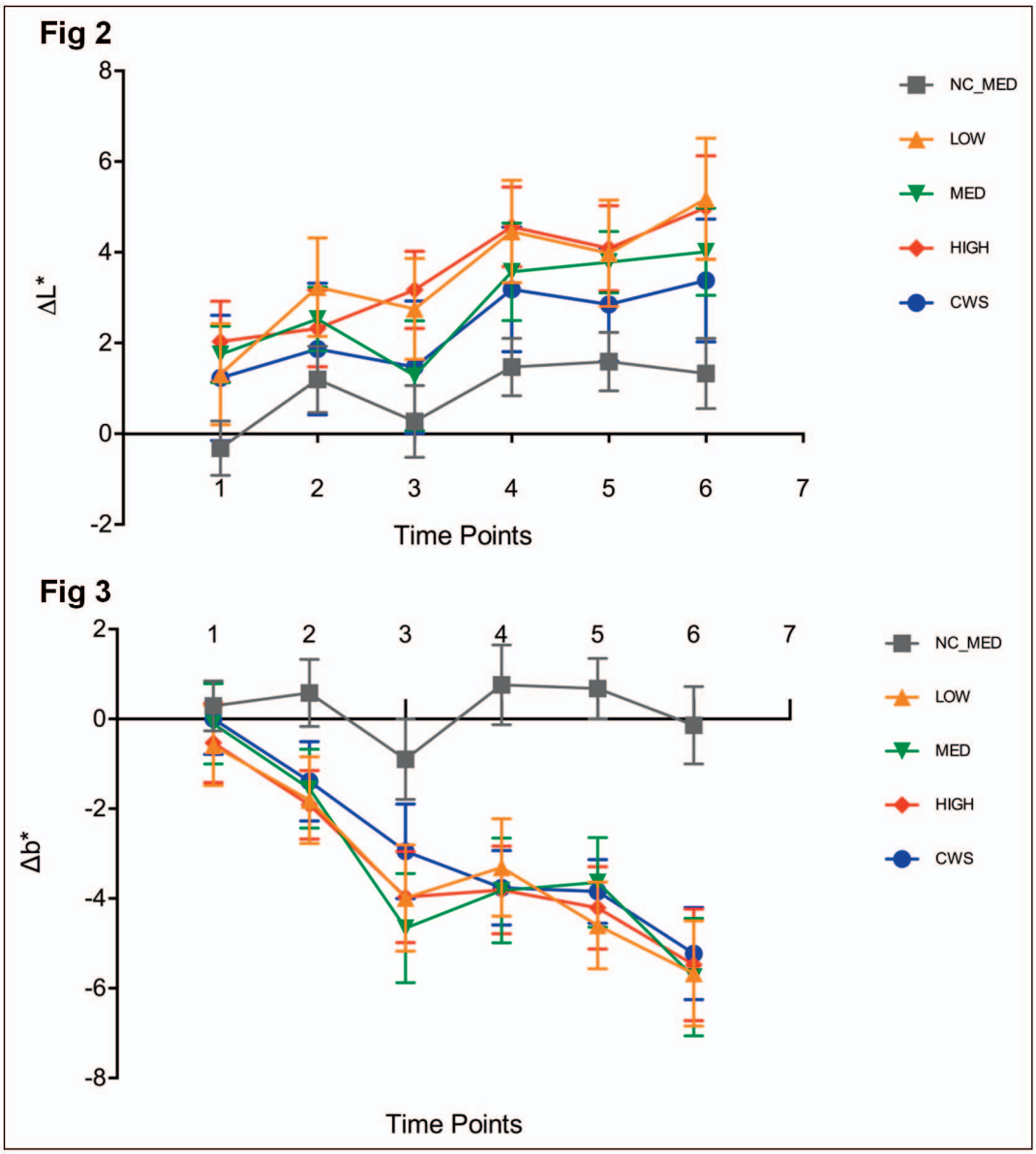

Figure 2. *Line plot of change in lightness* over time by group.*
Figure 3. *Line plot of change in the yellow-blue axis* over time by group.*

HP Penetration

The data provided strong evidence of differences in the distribution of HP penetration among the five groups ($p<0.001$). After we adjusted for multiple comparisons using an overall 0.05 level of type I error, lower HP values were observed for groups NC_MED (median: 0.24 μg/mL), HIGH (0.35 μg/mL), and CWS (0.36 μg/mL), which did not differ from each other ($p>0.05$). Higher HP values were observed for LOW (median: 0.48 μg/mL) and MED (median: 0.44 μg/mL), which were not different from each other, although each differed significantly from the other three groups ($p<0.05$). Figure 5 illustrates the distribution of HP penetration by group by box plots.

Viscosity

Figure 6 shows the average sample viscosity (η) for NC_MED, LOW, MED, and HIGH, plotted against the applied shear rate, $\dot{\lambda}$. All samples show shear thinning behavior described approximately as $\eta \sim \dot{\lambda}^{-0.8}$, with end-point viscosities (viscosity at the highest applied shear rate) of 6.64 Pa·s, 2.16 Pa·s, 4.0 Pa·s, and 14.4 Pa·s for NC_MED, LOW, MED, and HIGH, respectively. The increasing order of

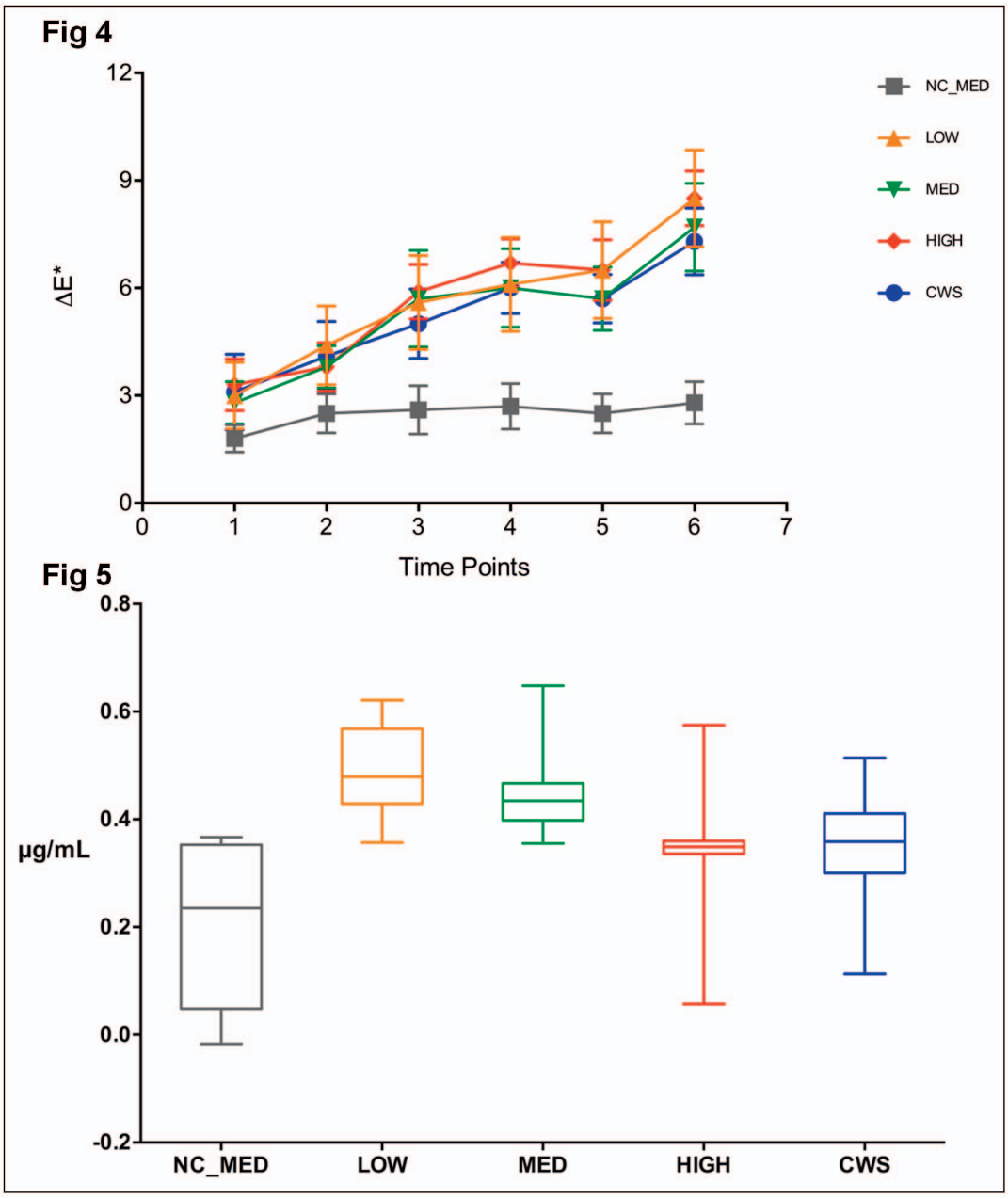

Figure 4. *Line plot of overall color change* over time by group.*
Figure 5. *Boxplots of HP penetration levels (µg/mL) by group.*

sample viscosities for groups LOW, MED, NC_MED, and HIGH can be observed over all shear rates investigated.

DISCUSSION

The oral health care industry is flourishing with a variety of whitening formulations and delivery systems. Nonetheless, the quest for the ideal material demonstrating maximum whitening with minimal adverse effects is ongoing. The current study compared bleaching gels of different viscosities for whitening efficacy and penetration potential into the pulp cavity. The rationale underlying this study was to provide information on the optimum viscosity and delivery system for developing future whitening strategies.

The findings supported our first null hypothesis that there would be no differences in color change among the experimental groups for ΔL*, Δb*, and ΔE* at any time points. Though the results were not

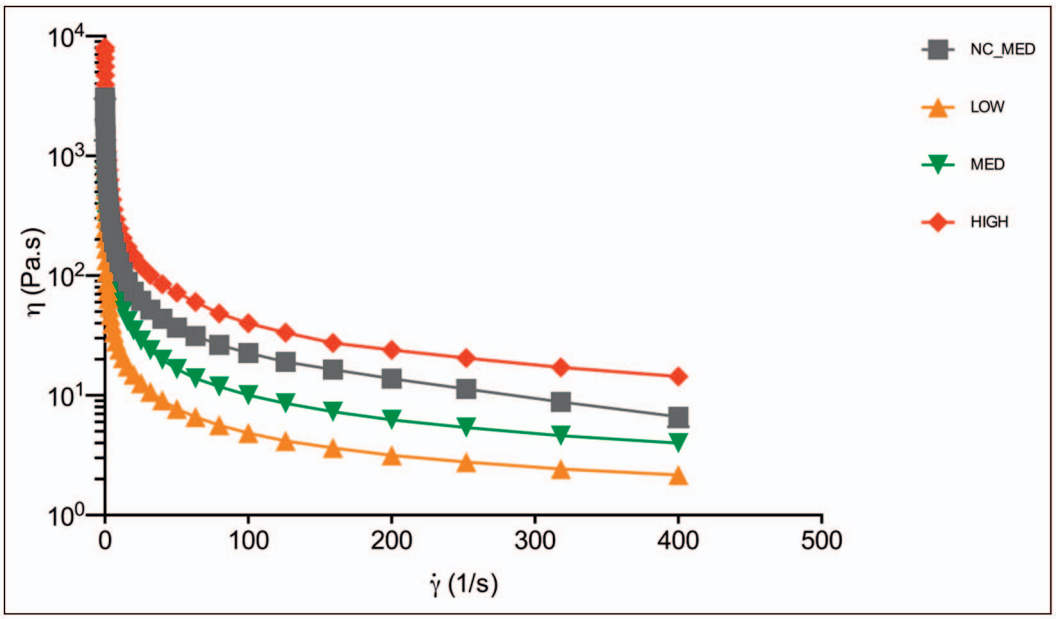

Figure 6. *Line plot of shear rate to viscosity by group.*

surprising, our study was the first to show that viscosity and type of delivery system did not affect whitening efficacy when concentration and exposure time were constant. We used the 50% acceptability threshold of $\Delta E^*=2.7$ to interpret tooth color change results.[12] Professionally dispensed, at-home whitening methods with the ADA Seal of Acceptance continue to use 10% carbamide peroxide in custom-fabricated trays with exposure times of up to 8 hours have shown long-term success rates in terms of efficacy and safety.[13-16] However, it is imperative to note that the trend in the over-the-counter (OTC) market is to promote higher concentrations at shortened exposure times.[17-21] We used 10% HP with an exposure time of 1 hour to relate to instructions of OTC products. According to the International Organization for Standardization (ISO 28399: 2011), a test method for laboratory assessment of whitening efficacy, ΔE^* should be two or greater for the product to be regarded as acceptable.[22] The standards do not indicate a post-measurement time point, but all four experimental groups in this study surpassed the efficacy requirement with simply a single application at 1 day after whitening.

It is evident that HP needs to diffuse from the enamel into the dentin to exert its effect on stain molecules. There are numerous *in vitro* studies quantifying HP penetration under different circumstances.[3-8,23] Still, considering the potentially toxic effects that HP has on the pulp, it is debatable whether a high penetration is desirable for superior whitening efficacy.[24] Attention to the rheological properties of whitening gels was given about withstanding shear stress so that it does not flow out of the tray and is not easily washed away or swallowed.[25] However, there have been no studies relating possible relationships between HP composition and viscosity of the whitening material.

Based on the results, our second null hypothesis was rejected. Notably, there was a difference among the experimental groups with lower viscosity gels (LOW and MED) showing higher penetration levels than the high viscosity gel (HIGH) and the strip delivery system. This is in accordance with another study that evaluated the penetration potential of potassium nitrate, which is a commonly used agent to prevent and manage tooth-whitening-induced sensitivity. The study found that potassium nitrate penetration was influenced by concentration and partly affected by the viscosity and suggested considering these properties for desensitizing formulations.[26]

Penetration studies are limited by not fully representing the dynamic *in vivo* process with a positive pulpal pressure during the whitening process. Additionally, the pulp cavity was enlarged to hold the acetate buffer required to stabilize HP in the pulp cavity. Even so, the current study provided relevant data on the relationship of rheological properties and type of delivery system to whitening efficacy and HP penetration. Future studies are

needed to evaluate the significance of these rheological properties on adverse effects such as gingival irritation, as the penetration of HP into gingival tissue is expected to differ based on viscosity. These findings could inform future strategies for developing whitening formulations with minimal adverse effects and maximum efficacy.

CONCLUSIONS

Within the limitations of this *in vitro* study,

1. Tooth whitening efficacy is not influenced by the rheological properties of the gel or delivery system when exposure time and concentration are kept constant.
2. The level of HP penetration is affected by the viscosity of the material, with lower viscosity materials exhibiting higher penetration levels in the pulp cavity.

Acknowledgements

The authors thank Ultradent Products Inc for kindly providing all bleaching materials for this study and Ms Alaina Piper for designing the diagrams.

Regulatory Statement

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

Conflict of Interest

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

(Accepted 30 May 2017)

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Conservative Treatment of a Complicated Crown-root Fracture Using Adhesive Fragment Reattachment and Composite Resin Restoration: Two Year Follow-up

AV Martins • RC Albuquerque • LD Lanza • ÊL Vilaça • NRFA Silva • AN Moreira • RR da Silveira

Clinical Relevance: Tooth fragment reattachment associated with composite resin restoration can be an excellent treatment option for complicated crown-root fracture, when one of the fragments has been lost. A multidisciplinary approach can be critical for success.

doi: http://dx/doi.org/10.2341/15-219-S

Effectiveness of the Multilayered Caries Model and Visuo-tactile Virtual Reality Simulator for Minimally Invasive Caries Removal: A Randomized Controlled Trial

AP Dwisaptarini • S Suebnukarn • P Rhienmora • P Haddawy • S Koontongkaew

Clinical Relevance: Differentiation and selective removal of carious dentin are important in minimally invasive caries treatment. The virtual reality simulator and multilayered model of a carious tooth reconstructed from micro-CT images representing the infected and affected carious layer with different colors and hardnesses provide visuo-tactile sensation for training in minimally invasive caries removal.

doi: http://dx/doi.org/10.2341/17-083-C

Mechanical and Surface Properties of Monolithic Zirconia

LM Candido • LN Miotto • LMG Fais • PF Cesar • LAP Pinelli

Clinical Relevance: Restorations produced with monolithic zirconia are frequently used to replace those made with conventional zirconia and veneering porcelain. However, for correct use, it is important to know key material features, such as mechanical strength and fractographic behavior.

doi: http://dx/doi.org/10.2341/17-019-L

Compliance of Randomized Clinical Trials in Noncarious Cervical Lesions With the CONSORT Statement: A Systematic Review of Methodology

A Reis • JL de Geus • L Wambier • M Schroeder • AD Loguercio

Clinical Relevance: Systematic reviews are the top level of evidence, and the results may help clinical decisions that are needed to provide the best treatment for patients. In face of that, the adherence of randomized clinical trials evaluating adhesive systems should be improved.

doi: http://dx/doi.org/10.2341/17-060-L

Diagnosis of Pit-and-fissure Caries Using Three-dimensional Scanned Images

JK Mitchell • AR Furness • RJ Sword • SW Looney • WW Brackett • MG Brackett

Clinical Relevance: Detecting occlusal caries with scanned three-dimensional images is as accurate as visual examination with magnification and appropriate lighting.

doi: http://dx/doi.org/10.2341/17-076-L

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Conservative Treatment of a Complicated Crown-root Fracture Using Adhesive Fragment Reattachment and Composite Resin Restoration: Two Year Follow-up

AV Martins • RC Albuquerque • LD Lanza • ÊL Vilaça
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Clinical Relevance

Tooth fragment reattachment associated with composite resin restoration can be an excellent treatment option for complicated crown-root fracture, when one of the fragments has been lost. A multidisciplinary approach can be critical for success.

SUMMARY

Crown-root fracture is one of the most challenging fracture types in the dental traumatology literature. Traumatized anterior teeth require quick functional and esthetic repair. In the case of a complex crown fracture of the maxillary left central incisor, requiring endodontic treatment, a fiber-reinforced post was used to create a central support stump to restore the dental morphology. This report describes the clinical procedures involved in the treatment. After two years of follow-up, the clinical and radiographic findings demonstrated that the adopted clinical protocol was successful and yielded healthy periodontal tissues with no signs of periradicular pathology.

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DOI: 10.2341/15-219-S

INTRODUCTION

Traumatic dental injuries are a common dental health problem and can result in damage to dental and periradicular structures, producing physical and psychologic discomfort, causing pain, and having a substantial impact on quality of life.[1-3]

Traumatic injury to teeth and their supporting structures usually occurs in young people, and maxillary central incisors are the most commonly affected teeth in either permanent or primary dentition because of their exposed position in the dental arch.[4] A crown-root fracture is a type of dental trauma, usually resulting from horizontal impact, that involves enamel, dentin, and cementum, occurs below the gingival margin, and may be classified as complicated or uncomplicated, depending on whether pulpal involvement is present or absent.[5-7]

The prognosis of traumatic injuries depends on early intervention to injured teeth and the extension of the intervention. A delay in treatment may influence the diagnostic results. There are many treatment modalities to treat teeth with a complicated crown-root fracture, depending on fracture location. It has been recommended that all involved fragments be removed to evaluate the extent of the injury.[8,9] Restoration of a tooth with a crown-root fracture or a cervical root fracture is unfavorable and can be a difficult procedure when the fracture line extends below the marginal bone level. Restorative and functional needs are balanced with the demands for a healthy periodontium.[10,11] Placing the margin of the restoration in the biologic width frequently leads to chronic gingivitis, the loss of clinical attachment, bony pockets, and gingival recession. Crown-root fractures extending well below the alveolar crest can require surgical repositioning of the tissues to expose the level of the fracture. Either surgical or orthodontic extrusion can also be performed to allow for better restoration of the fractured tooth. The choice of treatment is primarily determined using exact information about the site and the type of fracture, but the cost and complexity of treatment can also be deciding factors.[9-12]

This report describes a multidisciplinary approach for the treatment of a complicated crown-root fracture of a maxillary central incisor, with two years of follow-up.

CLINICAL CASE REPORT

A 23-year-old male patient came to the Dental School of Minas Gerais Federal University after falling and suffering a traumatic injury to the left central incisor two weeks before (Figure 1A,B). He reported that he broke his tooth while playing soccer. The medical history was reviewed, and there was no remarkable report. A written informed consent form was signed by the patient for treatment and further publication of the case.

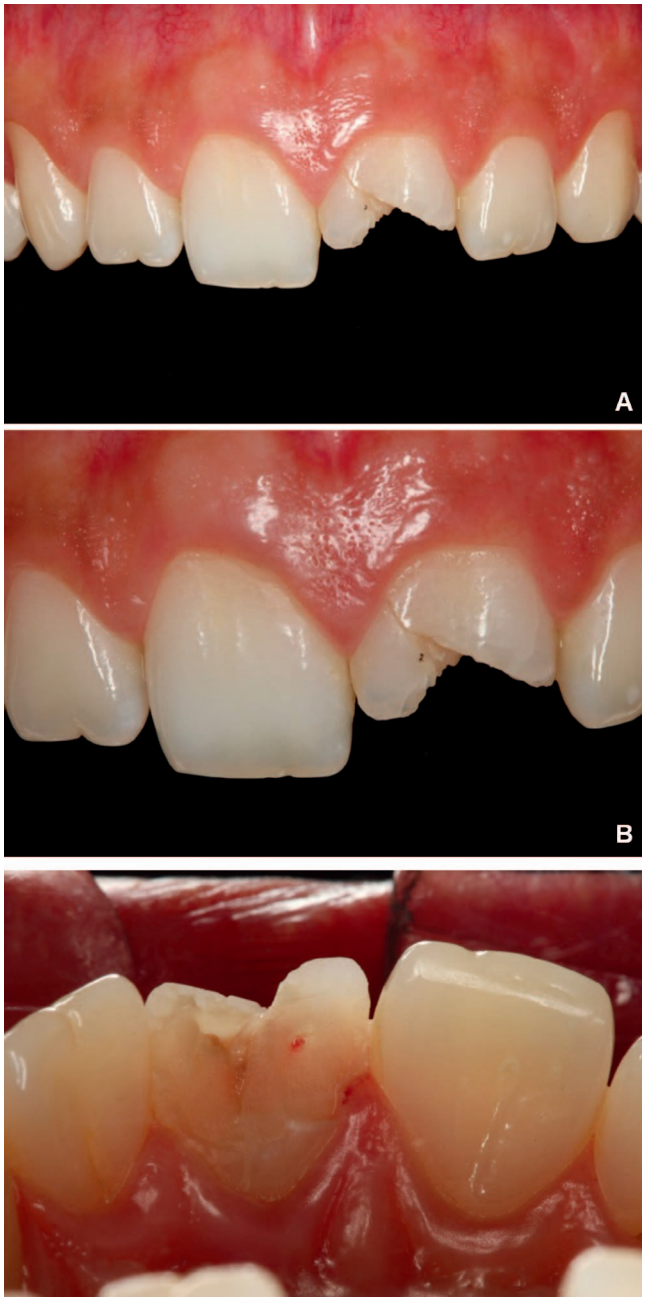
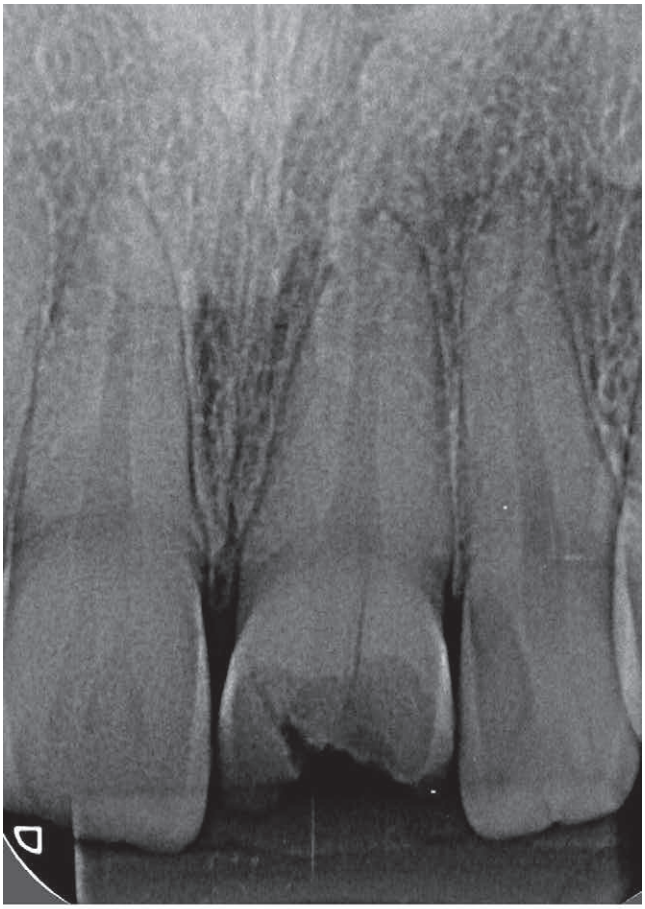


Figure 1. *(A) Intraoral view of the patient before the treatment. (B) Close-up view of the fractured tooth.*
Figure 2. *Palatine view.*

The clinical examination revealed a significant loss of tooth structure, pulp exposure, and a



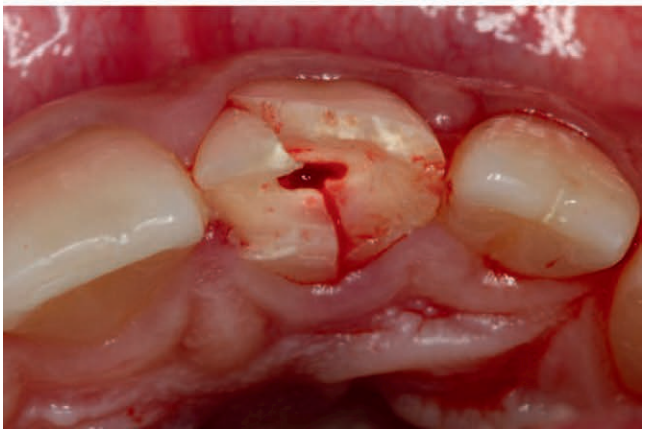


Figure 3. *Periapical radiograph of the fractured tooth.*
Figure 4. *Clinical aspect of the fragment still stuck to the gum fibers.*

horizontal coronary fracture affecting the mesiodistal surface of the left central incisor (Figure 2). Intraoral periapical radiographic investigation revealed the presence of a longitudinal crown-root fracture and showed no signs of bone fracture (Figure 3). The tooth had a two-part crown fracture: one of the fragments was lost at the scene of the accident and the other fragment was still in place, held by the gingival tissue. A coronal opening was made with removal of the entire pulp tissue to prevent contamination of the area that was to receive the fragment. The lingual fragment was mobile in the lateral direction, and the fracture extended subgingivally, with invasion of the periodontal biologic space (Figure 4).

After routine collection of the patient's dental/medical history and examination, a treatment plan was established. Because of the loss of one of the crown fragments, the proposed treatment was surgery for augmentation of the remaining clinical crown, the reattachment of the retrieved fragment, endodontic treatment, and a fiberglass post associated with composite resin restoration.

Under local anesthesia, an intrasulcular incision was made on the palatal gingival tissue of both maxillary central incisors and the maxillary left lateral incisor with a no. 15 scalpel blade for removal of the displaced fragment and exposure of the fracture line (Figure 5). In this case, the vertical difference between the alveolar bone crest and the fracture line was 1 mm (ie, there was a violation of the biologic space; Figure 6). Then, osteotomy and osteoplasty were performed, so that the fracture line stayed at a 2-mm bone margin. The coronal fragment (Figure 7) was rehydrated by immersing in normal physiologic saline solution and cleaned to remove foreign debris prior to the absolute isolation of the operative field (Figure 8).

After rubber dam isolation, again the fragment was positioned to check its perfect adaptation to the remaining structures (Figure 9). The enamel surface and both the remaining tooth structure and the fractured segment were etched with 37% phosphoric acid for 30 seconds. Following the etching, the etchant was removed by washing for 60 seconds, and the excess water was removed using paper towels. The fragment was adapted and reattached with resinous auto-adhesive cement. All margins were light cured for 40 seconds (Figure 10), and all subgingival margins were polished using a composite polishing kit (Astropol Composite polishing kit - Ivoclar Vivadent, Liechtenstein).

A commercially prepared antibiotic–corticosteroid product was placed in the canal space. The tooth was provisionally sealed with glass ionomer cement. The rubber dam was removed, and suturing was performed. An alginate impression and plaster model were used for establishing the contours of the maxillary left central incisor with diagnostic waxing (Figure 11). After seven days, the suture was

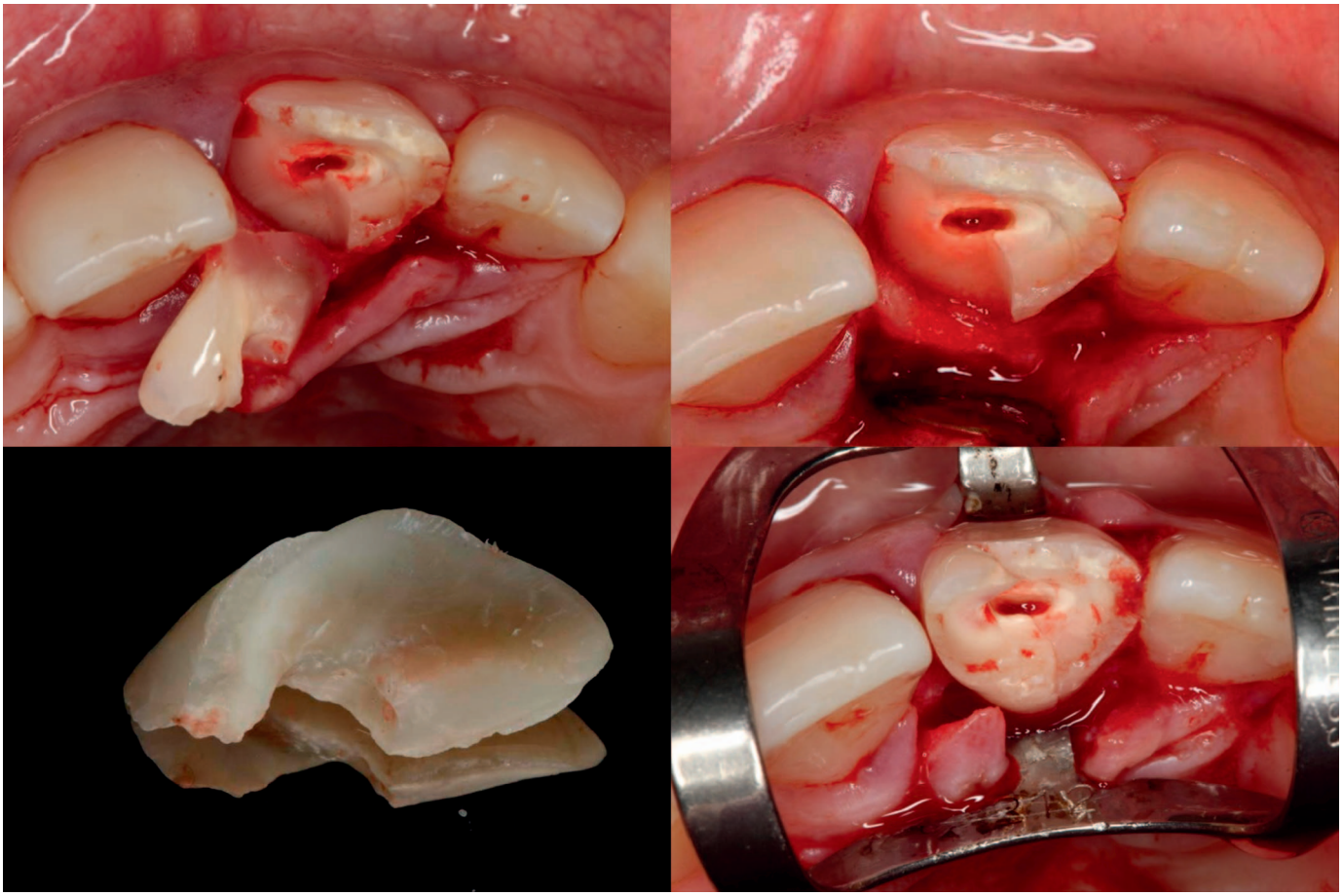


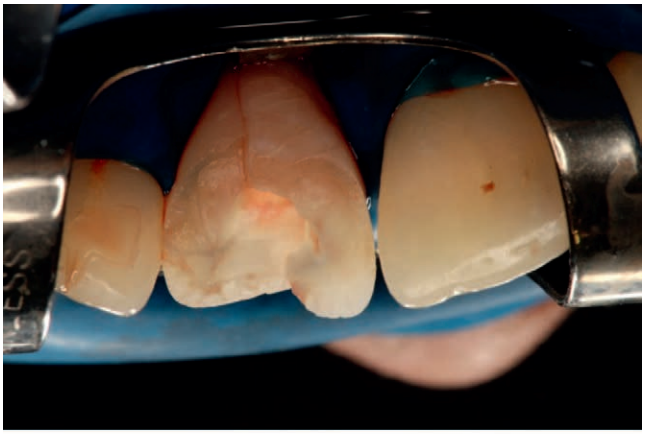
Figure 5. *Fragment being shifted.*
Figure 6. *Clinical aspect showing the extension of the complicated crown-root fracture, invading the biologic width.*
Figure 7. *Tooth fragment.*
Figure 8. *Retractor clamp and fragment positioned, certifying the perfect adaptation to the remaining dental structure.*

removed, and the patient was referred for endodontic therapy. Two days later, total removal of pulp tissue from the remaining root portion was accomplished under copious 1% NaOCl irrigation and with a Ni-Ti rotary instrument. After instrumentation, the canals were filled with 17% EDTA for three minutes, flushed with saline, and dried with absorbent paper points. Root canal obturation was performed with a thermoplastic obturation technique–System B plus canal sealer. The endodontic therapy was completed in one session.

The patient was scheduled to return at 90 days after reestablishment of the biologic width to perform the final restoration.

A silicone guide was created, from the diagnostic waxing, to assist the intraoral reconstruction with composite resin. After removal of the temporary restoration of glass ionomer cement, the guide was held to the occlusal contacts in the maximum intercuspation and disocclusion guides (Figure 12). Under a rubber dam, the treatment continued with post space preparation. A previously selected fiberglass post was used. This was reduced coronally, taking into account the previously positioned silicone guide (Figure 13). The radicular portion was washed with distilled water and then dried. The post was cemented inside the root canal with resinous autoadhesive cement, according to the manufacturer's instructions. The coronal portion corresponding to the fragment lost during the traumatic injury was built with composite resin, using the silicone guide previously created. The restoration was adjusted, respecting the contacts previously made. After 20 days, the restoration was polished using diamond stones and a composite polishing kit. Occlusion was checked and adjustments were made as necessary. Then, the restoration was completed (Figures 14, 15 and 16).

All materials used are listed in Table 1.



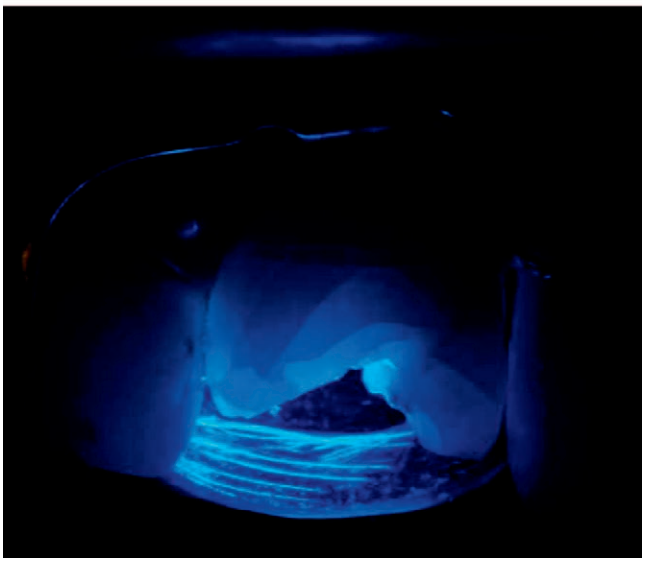
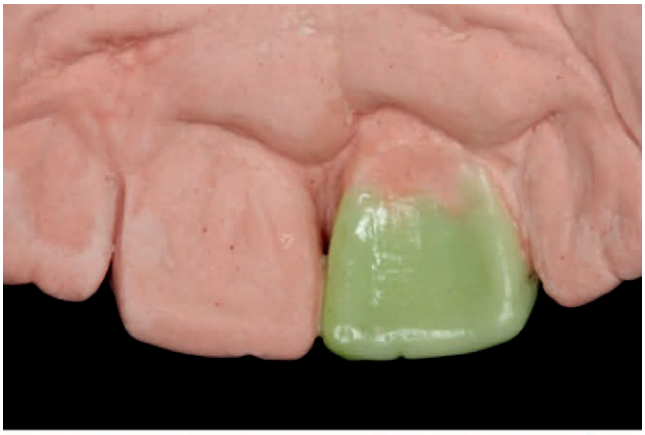


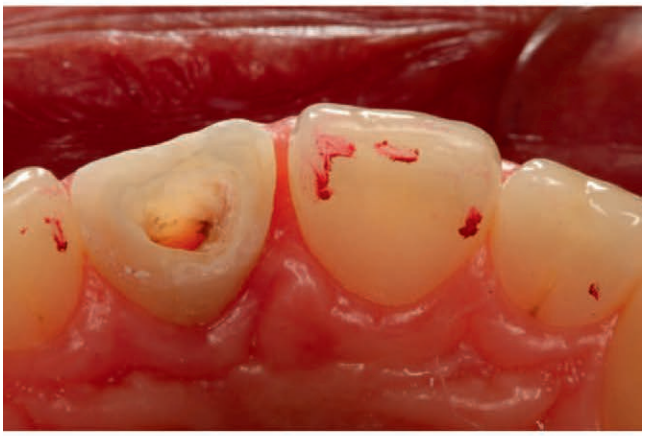
Figure 9. *Perfect fragment adaptation to the remaining dental structure.*
Figure 10. *Set fragment/remaining dental structure light cured for 40 seconds.*

After two years of follow-up, clinical examination showed good function and esthetics for the restored tooth.

POTENTIAL PROBLEM

The main causes of traumatic dental injuries reported in the literature are violence, collisions, falls, sports, leisure activities, and traffic accidents. Male individuals suffer significantly more traumatic dental injuries in the permanent dentition than females, probably because they are more frequently engaged in physical activities involving physical contact.[1] Additionally, the literature suggests that most traumatic dental injuries involve the maxillary central incisors, followed by maxillary lateral incisors and mandibular incisors. The prominent and open position of the upper teeth in the face is





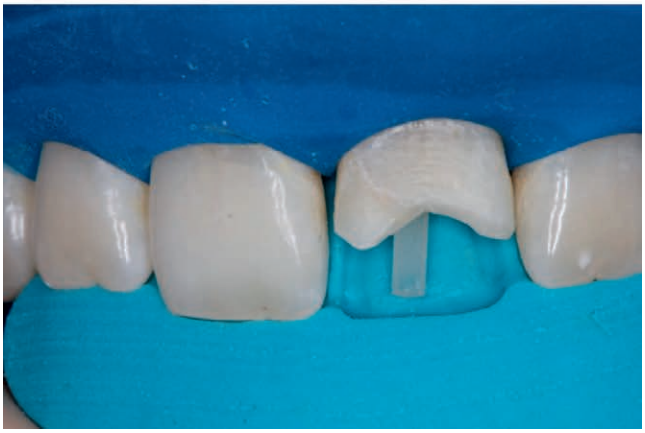


Figure 11. *Diagnostic waxing.*
Figure 12. *Record of occlusal contacts before restoration.*
Figure 13. *Fiber post with your specified length, from restoration dimensions previously planned.*

responsible for their more frequent involvement in fractures than the lower teeth.[1-4,13]

Crown fracture restorations localized in the superior incisor area need to be evaluated from several perspectives, including the topography, tissues involved, quality and the quantity of the remaining tooth structures, adaptation of the fragment to the

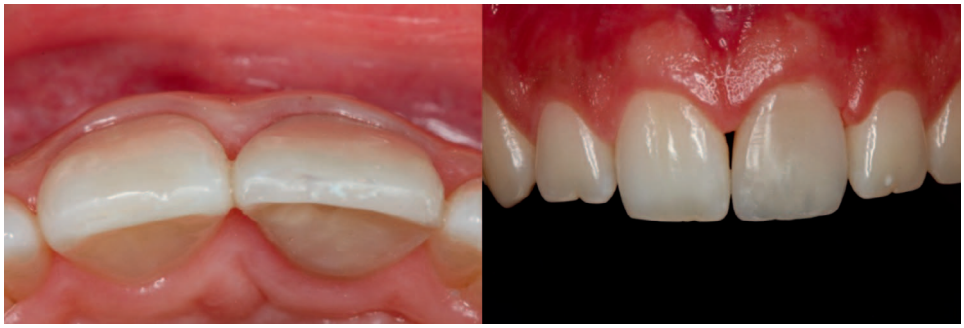


Figure 14. *Restoration completed: incisal view.*
Figure 15. *Restoration completed: anterior view.*

dental remnant, and the patient's age.[13-15] The choice of clinicians regarding the restorative treatment of fractured teeth directly affects the treatment prognosis and requires a careful consideration of several factors, such as the extent and pattern of the fracture, the endodontic and periodontal involvement, and the possibility of using the fragment in the reattachment process.[15,16]

A study of the literature shows that coronal restoration of teeth with crown-root fractures is usually challenging, especially when the fracture extends below the bone level, as occurred in the present case.[9,13,15,16] One of the determinant factors for the functional and esthetic success in the management of complicated crown-root fractures is the adoption of a multidisciplinary approach involving surgery, endodontics, periodontics, and prosthodontics.[9,13,16,17] The reconstruction of extensively destroyed anterior teeth has become a true challenge for restorative dentistry because dental materials do not effectively substitute for dental tissues. According to this case, two techniques are possible for treatment of complicated crown-root fractures, and the advantages and disadvantages of these techniques are listed in Table 2.

Root treatment carried out in a single visit is preferable in trauma cases such as this; the prognosis is extremely good for a vital pulp extirpation. In a case with a necrotic pulp or if the tooth has already been root treated, an evaluation of the individual tooth should be made to determine whether one or more appointments are appropriate.[3]

Table 1: *Materials Used and Commercial Brands*

Materials	Supplier
Rubber dam	SSWhite, Rio de Janeiro, RJ, Brazil
Phosphoric acid 37%	Dentalville do Brasil LTDA, Joinville, SC, Brazil
212 Retractor clamp	SSWhite, Rio de Janeiro, RJ, Brazil
Rely-X Unicem	3M/ESPE, St. Paul, MN, USA
Astropol Composite polishing kit	IvoclarVivadent, Liechtenstein
Otosporin	Farmoquímica S/A, Rio de Janeiro, RJ, Brazil
Vidrion R	SSWhite
EDTA	Odahcan-Herpo Produtos Dentários Ltda, Rio de Janeiro, RJ, Brazil
Filtek Z350XT	3M/ESPE, Ribeirão Preto, SP, Brazil
Whitepost Fiber post	FGM, Joinville, SC, Brazil

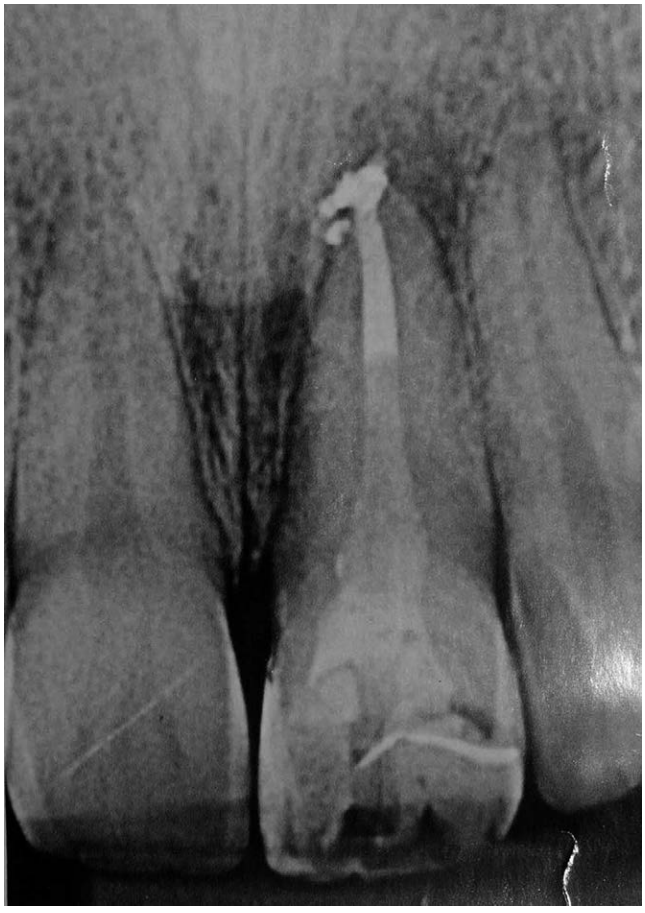


Figure 16. *Radiographic view after intra-radicular fiber post cementation and composite resin reconstruction.*

Table 2: *Advantages and Disadvantages for Each Technique*

Techniques	Advantages	Disadvantages
Tooth fragment reattachment associated to composite resin restoration	• Maintenance of dental substrate • Insertion of periodontal fibers in natural structure • Reduced cost • Fewer clinical sessions	• Possibility of remaining color change • Superficial staining of the composite resin
Ceramic crowns	• Color stability • Surface smoothness • Longevity	• Increased wear of tooth structure • Higher costs • Need for a greater number of treatment sessions • Need for orthodontic traction and additional surgeries

The technique of tooth fragment reattachment has advantages over direct composite resin restorations, namely, procedural simplification, less clinical chair time, and immediate reestablishment of aesthetics and function.[9-13,15] However, in this case, one of the fragments had been lost during the traumatic injury, which determined the need for associating the reattachment technique with a composite resin restoration.

With the adhesive materials and composite resins available today, in combination with an appropriate technique, esthetic results can be achieved with predictable outcomes of crown reattachment if complicated crown-root chisel-type fractures of the anterior teeth have occurred, especially in younger patients.[11,16,18] The retention of the restored portion and fragment to the masticatory effort was compensated for by the cementation of an intracanal post with fiber-reinforced resin posts. Such resin posts have been suggested as a group of materials that offers stiffness equal to that of dentin, as well as high durability and, therefore, have some esthetic advantages over metal posts.[19] A modulus of elasticity similar to that of dentin may increase the strength of the remaining tooth structure and reduce the risk of tooth fractures.[13]

Traumatic injuries can require a multidisciplinary treatment approach. The combined use of adhesive materials and a tooth fragment is a simple, low cost, and efficient procedure for the treatment of traumatized anterior teeth. This report provides a highly conservative approach that combines the esthetics, function, and health of periodontal tissues, postponing the use of a more aggressive prosthetic solution.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Dental School of Minas Gerais Federal University, Brazil.

Conflict of Interest

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

(Accepted 22 December 2015)

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Effectiveness of the Multilayered Caries Model and Visuo-tactile Virtual Reality Simulator for Minimally Invasive Caries Removal: A Randomized Controlled Trial

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Clinical Relevance

Differentiation and selective removal of carious dentin are important in minimally invasive caries treatment. The virtual reality simulator and multilayered model of a carious tooth reconstructed from micro-CT images representing the infected and affected carious layer with different colors and hardnesses provide visuo-tactile sensation for training in minimally invasive caries removal.

SUMMARY

This work presents the multilayered caries model with a visuo-tactile virtual reality simulator and a randomized controlled trial protocol to determine the effectiveness of the simulator in training for minimally invasive caries removal. A three-dimensional, multilayered caries model was reconstructed from 10 micro-computed tomography (CT) images of deeply carious extracted human teeth before and after caries removal. The full grey scale 0-255 yielded a median grey scale value of 0-9, 10-18, 19-25, 26-52, and 53-80 regarding dental pulp, infected carious dentin, affected carious dentin, normal dentin, and normal enamel, respectively. The simulator was connected to two haptic devices for a handpiece and mouth mirror. The visuo-tactile feedback during the operation varied depending on the grey scale. Sixth-year dental students underwent a pretraining assessment of caries removal on extracted teeth. The students were then ran-

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DOI: 10.2341/17-083-C

domly assigned to train on either the simulator (n=16) or conventional extracted teeth (n=16) for 3 days, after which the assessment was repeated. The posttraining performance of caries removal improved compared with pretraining in both groups (Wilcoxon, $p<0.05$). The equivalence test for proportional differences (two 1-sided *t*-tests) with a 0.2 margin confirmed that the participants in both groups had identical posttraining performance scores (95% CI=0.92, 1; $p=0.00$). In conclusion, training on the micro-CT multilayered caries model with the visuo-tactile virtual reality simulator and conventional extracted tooth had equivalent effects on improving performance of minimally invasive caries removal.

INTRODUCTION

Dental caries is the localized destruction of susceptible dental hard tissues by acidic by-products of bacterial fermentation of dietary carbohydrates.[1] According to GV Black in 1908,[2] the science of cariology and the technicalities of operative dentistry should be integrated in teaching and research of caries treatment. Dentistry in the 20th century shifted toward a minimal intervention approach, leading to changes on how and when to treat caries. The concept includes the use of all available information and techniques ranging from accurate diagnosis of caries, caries risk assessment, and prevention to technical procedures in repairing restorations.[3] The disease should be treated first; the surgical approach should be undertaken only as a last resort and then with the removal of as little natural tooth structure as possible. The endpoint of caries excavation in cavity preparation is to remove infected carious dentin as selectively as possible, while preserving affected carious dentin.[4] Avoiding over-excavation will preserve remaining tooth structure and offer longevity of restorations.[5] Unlike management of superficial or moderate caries lesions, managing very deep lesions requires more delicate skills. Conserving tooth structure and preserving pulp vitality contribute to a longer and better prognosis by avoiding pulp exposure as well as providing optimum bonding strength for adhesive restorations. In the symptomless case of a deep carious lesion, removing all infected dentin in an area that will probably expose the pulp should be avoided. Sealing the lesion will significantly decrease the number of bacteria by removing their nutritional sources.[6-8]

Treatment of dental caries, like other disciplines of dentistry, can be associated with unwanted or unforeseen procedural errors. The first step toward increasing patient safety in dental treatment is for all clinicians to acquire knowledge and skill in the early stage of training. Current practice in caries removal starts with gaining access through the enamel to the softened, infected carious dentin. The dentinoenamel junction (DEJ) is cut further until hard—and in some dental schools—stain-free dentin is reached. The end point of caries removal close to the pulp varies according to the country, dental school, and dentist.[9] Dental students are usually taught to assess dentin texture only by the touch of the dental probe. The dentin's darkening color, sensitivity, and wetness are useful but difficult to recognize.[10] Currently, skill acquisition in caries treatment follows an apprenticeship approach, which consists of close, expert supervision in preclinical settings and transfer of these skills to the clinic. This method of training may subject patients to discomfort, risk of complications, and prolonged procedure times, creating a clinical governance dilemma. At the same time, there may be limited access to apprenticeship training in more complex scenarios, with corresponding difficulty in training the student in a time-effective manner.

With the significant and consistent development of medical imaging and virtual reality (VR) research, minimal intervention training in dentistry should move toward more interactive and realistic environments. Using microcomputed tomography (micro-CT) permits construction of three-dimensional (3D) images of tooth and bone structural parameters from computerized axial tomography in the order of micrometers.[11] VR and image-processing programs incorporate 3D images obtained from CT scanners and transform them into patient-specific anatomic models that provide human-computer interaction with the manipulation of realistic environments.[12] Tactile sensation is important in dental skill acquisition, which can be achieved by incorporating haptic devices that allow users to interact with simulated oral tissues and dental instruments designed for each specific procedure in a virtual environment.[13] The users perform operations such as sensing, scraping, and cutting soft and hard tissues with realistic force feedback to the operator's hands. Several VR programs have been developed for dental application.[14-17] Recently, a multilayer model that mimics real tooth structures with different hardnesses was developed in a study for caries-removal training. The study aimed at caries-removal training

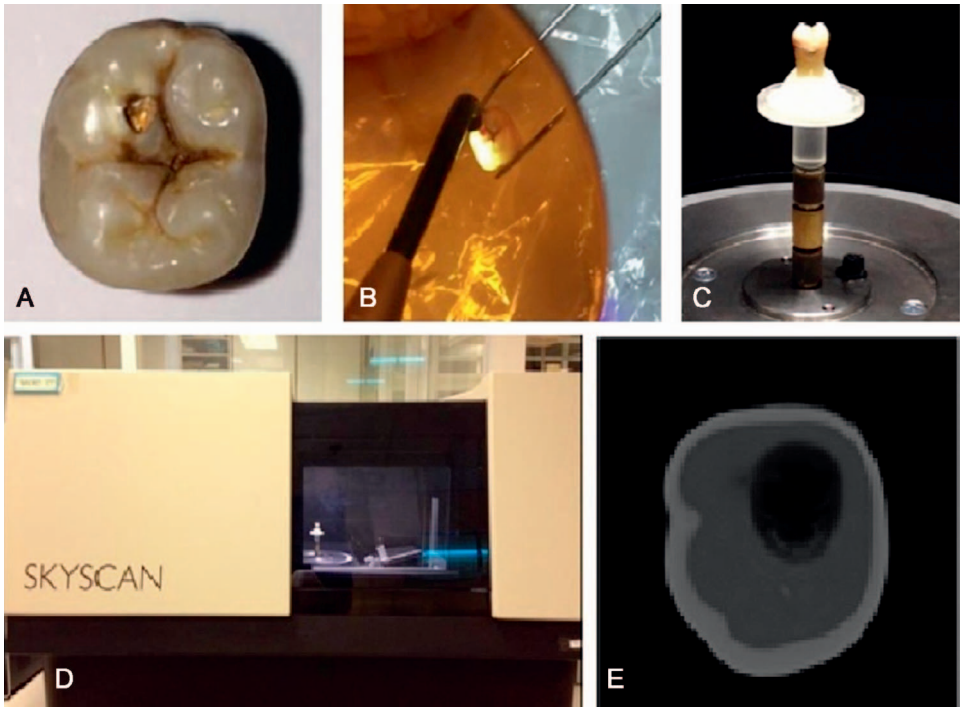

Figure 1. *Acquisition of micro-CT images. (A): Extracted permanent molars with deep carious lesion. (B): Minimally invasive caries removal guided by FACE. (C, D): Micro-CT scanning. (E): DICOM file slide No. 1630 showing infected carious dentin, affected carious dentin, normal dentin, and enamel.*

at repetitive training times.[18] However, these studies worked on shallow carious lesions with no difference between layers. The VR simulators allow students and experts to practice dental procedures anywhere and anytime with no incremental cost. The students trained by the VR system showed performance equal to or significantly greater than those trained by conventional methods.[14-17] Evidence as to the negative aspects of VR include initial setup costs, faculty training, and the lack of a variety of content and current educational simulation programs.[19] A study comparing the effectiveness of conventional training and VR simulation in operative dentistry training indicated that VR-based skills acquisition was unsuitable as the sole method of feedback and evaluation for novice dentists.[20]

Differentiation between infected and affected carious dentin is important in minimally invasive caries treatment. The goal is to remove infected carious dentin as selectively as possible through maximum preservation of affected carious dentin. To our knowledge, there is no VR- simulated carious tooth model that resembles a deep carious lesion likely to expose the pulp with infected and affected tissue in which caries removal needs to be done very carefully. This work presents a new, 3D, multilayered carious tooth model that reveals infected and affected carious dentin for the visuo-haptic VR simulator. Visual and haptic VR feedback, while cutting through each carious layer, varies depending on the micro-CT density. A randomized controlled trial protocol presented in this study aims to determine the stimulation's effectiveness when used as an alternative to conventionally extracted teeth for minimally invasive caries-removal training. We tested the hypothesis that there is no difference in performance between the conventional training group and the simulation training group.

METHODS AND MATERIALS

Multilayered Caries Modeling and Visuo-Haptic Virtual Reality Simulator

3D micro-CTs (SkyScan 1172; Bruker, Brussels, Belgium) of 10 extracted permanent molars with deep carious lesions were acquired before and after minimal caries removal guided by fluorescence-aided caries excavation (FACE; Sirona, Hanau-Wolfgang, Germany) (Figure 1); the original patient could not be identified. Micro-CT creates a 3D map of x-ray attenuation coefficient of materials within an object, corresponding to the x-ray opacity of an object. As a result, it is likely to separate various segments of a material from each other by density thresholding. Immersion of x-rays by hard- and soft-tissue layers of a tooth such as dental pulp and enamel increases with decreasing x-ray photon energy, and that relative difference in absorption also decreases with less x-ray energy. A grey scale value that micro-CT produces is a single number that represents the brightness of the pixel. The most common pixel

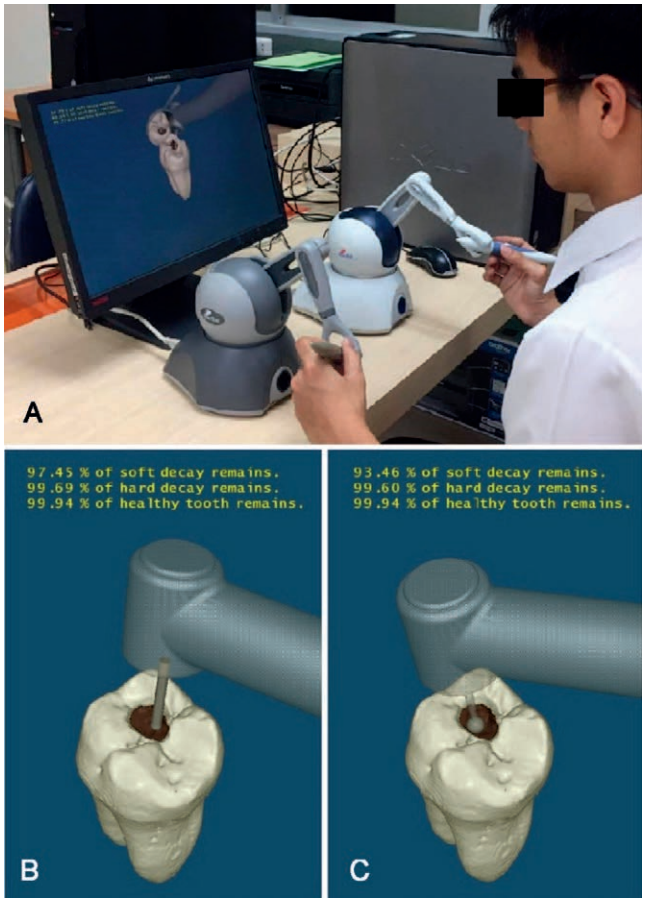

Figure 2. *The simulator (A) had a high-speed bur for cutting enamel (B) and slow-speed bur for removing carious dentin (C).*

format is the byte image, in which this number is stored as an 8-bit integer, giving a range of possible values from 0 to 255. Typically, zero is assumed to be black, and 255 is taken to be white.[21]

In this study, we used a semi-automated, slice-by-slice hand contouring approach, the current segmentation gold standard using 10 datasets of extracted permanent molars with deep carious lesions.[22] Snake algorithms based on large image gradients were used to recognize the contours of edges of interest.[23] The full gray scale 0-255 yielded a median gray-scale value of 0-9, 10-18, 19-25, 26-52, and 53-80 regarding dental pulp, infected (soft) carious dentin, affected (hard) carious dentin, normal dentin, and enamel, respectively.

3D reconstruction of each tooth was performed using 2500 two-dimensional images processed by the volume-rendering method. Segmentation of the multilayered, carious model selectively represented enamel, infected dentin, affected dentin, healthy dentin, and dental pulp. The model was generated by

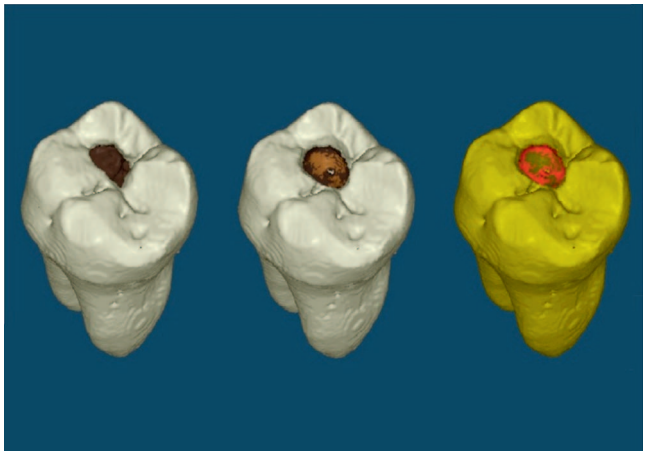

Figure 3. *3D models of extracted human teeth were reconstructed from micro-CT images. The infected carious dentin is represented as a darker, dull brown color, and the affected carious dentin is seen as a light brown color. The FACE mode that represents infected carious dentin is a red-fraction color layer.*

the subtraction process of the micro-CT images before and after the removal of caries.

VR operates on a 2.8-GHz Pentium 4 PC, with 256 MB RAM and a 13-inch computer monitor connected to two haptic devices. Two Omni haptic devices (SensAble, Inc, Woburn, MA, USA) for the handpiece and mouth mirror were used, which allowed 6° of freedom for positional sensing and generated 3° of freedom for force feedback.[24] The force feedback, while cutting through each carious layer, varied depending on the micro-CT density value. The number of volumetric sample points of the cutting tool model immersed into tooth voxels was detected. The immersed sample points indicating the depth penetrated were removed corresponding to the shape of the bur and the amount of force applied to the tooth surface. The simulator had a high-speed bur for cutting enamel and slow-speed bur for removing carious dentin. Visual feedback consisted of tooth deformation, color, and amount of tooth removed during the operation. The percentages of each carious layer remaining while cutting were displayed in real time (Figure 2). The infected carious dentin was represented as a darker dull brown color, and the affected carious dentin was seen as a light brown color imitating a natural carious tooth (Figure 3). The simulator also had FACE mode, representing infected carious dentin as a red-fraction color layer initiated by the porphyrin produced by the bacteria when lit by the system. A brownish color is seen when affected dentin is reached, similar to the presence of sclerotic dentin produced by chronic caries. The FACE mode ensured that all infected

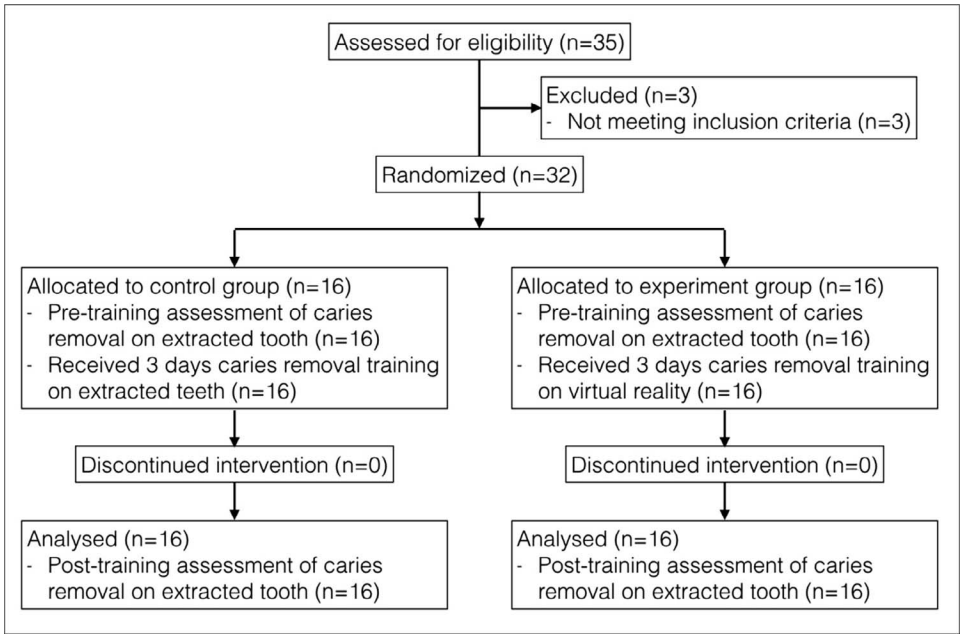

Figure 4. *Flow chart of participants in the randomized controlled trial.*

dentin was eliminated during the caries-removal procedure.

Randomized Controlled Trial of Efficacy for Minimally Invasive Caries-Removal Training

A prospective randomized controlled and blinded trial was conducted to test the hypothesis that training with the experimental group's multilayered caries model and visuo-tactile VR simulator will produce performance scores in caries-removal training similar to those obtained from conventional training with carious extracted teeth.

Sample Size

A continuous response variable from independent control and experimental participants with one control per experimental participant was studied. In a previous study,[25] the response within each participant group was normally distributed, with a standard deviation of 0.25—the true difference in the experimental and control means. As a result, 16 participants in each conventional training group and VR group were employed to reject the null hypothesis that the population means of the experimental and control groups are equal, with a probability of 0.8. The type I error probability associated with the test of this null hypothesis was 0.05. Sixth-year dental students were recruited. Exclusion criteria were as follows: 1) had experience with the simulator or 2) received a score below 70% in knowledge assessment of minimally invasive caries removal. All participants gave their written informed consent, which was approved by the institutional ethics review board.

Study Design

Participants were randomly assigned to either the experimental group (caries-removal training with micro-CT multilayered caries model and visuo-tactile VR simulator) or the control group (conventional training with carious extracted teeth). A statistician not involved with the study undertook the randomization using sealed opaque envelopes (Figure 4). The study had a pretraining/posttraining control group design. On pretraining day (day 1), each participant was asked to perform minimally invasive caries removal on one extracted tooth. On training days (days 2, 3, and 4), 16 participants in the control group practiced caries removal on two extracted teeth, and 16 participants in the experiment group practiced caries removal on 2 micro-CT multilayered carious teeth using the visuo-tactile VR simulator. In the control group, the carious and undermined enamel was prepared with a cylindrical bur (Meisinger, Neuss, Germany) using a high-speed handpiece with water coolant until direct vision into the entire cavity was achieved. Caries removal on the peripheral walls and pulpal floor was performed using a tungsten carbide round bur with a low-speed handpiece. The goal was to eliminate the infected dentin and preserve the affected dentin, as confirmed by the FACE system. The training process was similar in the experimental group, in which caries removal was carried out in the simulator. On

Table 1. *Pretraining and Posttraining Performance Scores Between Caries Removal Training With Micro-CT Multilayered Caries Model and Visuo-Tactile VR Simulator (Experimental Group) and Training With Extracted Carious Teeth (Control Group) (Mean±SD)*

	Virtual Reality (Experimental) Group (n=16)		Extraction (Control) Group (n=16)	
	Pretraining	**Posttraining**	**Pretraining**	**Posttraining**
Enamel	4.64±2.54	6.64±1.57	5.00±2.93	6.23±1.57
DEJ	5.27±1.27	6.36±1.36	5.15±2.42	6.81±1.89
Superficial dentin	3.45±2.34	7.42±1.06	3.91±1.70	6.01±1.10
Deep dentin/pulpal floor	0.36±0.47	1.76±0.32	0.35±0.52	1.25±0.49
Total	13.23±2.50	20.41±2.56	13.36±2.08	19.09±2.51

posttraining (day 5), each participant was asked to perform minimally invasive caries removal on one extracted tooth similar to pretraining.

The main outcome measure in both groups was performance scores assessed by an expert blinded to trainee and training status. The secondary outcome measures were tooth mass loss and task completion time. To determine the performance score, three-point scales were applied to four walls (buccal, lingual, mesial, distal), judged in four areas (enamel, DEJ, superficial dentin, deep dentin/pulpal floor), with 0 defined as "infected dentin left/pulp chamber perforation," 1 as "overprepared or overexcavated enamel/reaching sound dentin," 2 as "no undermined enamel/no carious enamel." The total maximum performance score was 26. Each tooth's mass was measured and recorded in grams before and after the caries removal procedure on a digital analytical balance accurate to 0.0001 g. The percentage of tooth mass removed for each tooth was calculated. The total time taken to complete the task was measured to an accuracy of 0.01 minute.

Statistical Analyses

The Wilcoxon test was used for nonparametric data and matched pairs to examine differences between pretraining and posttraining caries-removal performance scores in the same group. The Mann-Whitney test was used for unmatched data to detect any differences between the experimental and control groups. The dependent *t*-test and independent *t*-test were used to compare the tooth-removal and task-completion time within and between the experimental and control groups. Statistical significance was defined as a p value less than 0.05. All analyses were undertaken using SPSS version 21.0 (SPSS, Inc, Chicago, IL, USA). Results between the experimental and control groups were compared using an equivalence test for proportional differences (2 one-sided t-tests, [TOST]) with a 0.2 margin. The TOST method effectively tests the hypothesis that two estimates are equivalent within a statistically computed equivalence range. The lower the p value, the more similar the estimates can be concluded to be, and if the p value is less than the critical cutoff (0.05), then the two estimates can be assumed to be statistically equivalent. Statistical tests with p values of less than 0.05 were considered significant.

RESULTS

Thirty-five sixth-year dental students were recruited. Three students were not admitted to the study due to their experience with VR simulation. None of the participants in the control or experimental groups dropped out before completing the posttraining assessment. Table 1 shows that the pretraining mean total performance scores of the experimental group (13.23±2.50) and control group (13.36±2.08) were almost identical. The Mann-Whitney test confirmed that there were no significant differences. The mean posttraining performance scores were significantly higher than the pretraining scores in both experimental (13.23±2.50 to 20.41±2.56) and control groups (13.36±2.08 to 19.09±2.51) (Wilcoxon, $p<0.05$), indicating that performance significantly improved after training in both groups. The average posttraining performance score for the experimental group (20.41±2.56) was not significantly different from that obtained for the control group (19.09±2.51), indicating that performance improved similarly in minimally invasive caries-removal training with the micro-CT multilayered caries model and visuo-tactile VR simulator (experimental group) and training with carious extracted teeth (control group).

The equivalence test for proportional differences confirmed that participants in both groups had identical posttraining performance scores (TOST, 95% CI=0.92, 1; $p=0.00$). However, comparing the mean performance scores in four areas (enamel, DEJ, superficial dentin, deep dentin/pulpal floor), the average posttraining performance score of

Table 2. *Tooth Mass Removed and Task Completion Time Between Caries Removal Training With Micro-CT Multilayered Caries Model and Visuo-Tactile VR Simulator (Experimental Group) and Training With Extracted Carious Teeth (Control Group) (Mean±SD)*

	Virtual Reality (Experimental) Group (n=16)		Extraction (Control) Group (n=16)	
	Pretraining	Posttraining	Pretraining	Posttraining
Tooth mass removed (g)	4.98±2.61	5.68±2.48	5.87±3.41	6.73±2.38
Task completion time (s)	14.90±7.14	10.82±4.17	14.18±7.29	10.87±4.47

superficial dentin for the experimental group (7.42±1.06) was significantly greater than that obtained for the control group (6.01±1.10). Similarly, the average posttraining performance score of deep dentin/pulpal floor for the experimental group (1.76±0.32) was significantly greater than that obtained for the control group (1.25±0.49). There were no differences in tooth mass removed and task completion time after training in both groups (Table 2).

DISCUSSION

Differentiation and selective removal of infected and affected carious dentin is important in training minimally invasive caries treatment. Traditional caries removal in restorative treatment aims to eliminate all softened parts of a cavity, which is assumed to be mandatory to terminate the caries process. Some dental schools still teach removal of all lesions until reaching stain-free and "hard," healthy tissue in the entire area.[26] However, caries progression does not always turn into worst-case scenarios such as pulpitis or pulp necrosis, despite leaving some areas of infected dentin. Many bacteria still exist in a cavity from which all soft dentin has been eliminated, regardless of the excavation system used.[27] Significantly lower organism activity would be achieved when active cariogenic mass and part of the demineralized dentin is removed; hence, excavation on a deep pulpal floor need not be aggressive.[28]

Several techniques have been developed for removing carious layers selectively, such as conventional slow-speed tungsten carbide bur, caries detector application, chemical excavation, laser, and fluorescence-aided caries excavation (FACE), which is a direct method to clinically differentiate between infected and affected carious dentin. Because several oral microorganisms produce orange-red fluorophores as by-products of their metabolism (porphyrins), infected carious tissue will fluoresce, especially in the red fraction of the visible spectrum due to the presence of proto- and mesoporphyrins.[29] One study has shown that the number of bacteria remaining after FACE excavation is statistically lower than after traditional or caries-detected excavation.[30]

In the current study, we developed a multilayered caries model for minimally invasive visuo-haptic simulation using micro-CT. Our model represented real dental materials such as enamel, sound dentin, infected dentin, affected dentin, and dental pulp with different mechanical hardnesses. Furthermore, the model delivered different colors for separating infected dentin from affected dentin as guided by the FACE system for selectively removing the carious layers: red for infected dentin and brown for affected dentin. The simulator considered caries-removal training in a minimal intervention manner. FACE prevented widening the cavity size significantly by removing infected dentin effectively compared with other systems.[31] We also presented high-speed or low-speed cutting with diamond or tungsten carbide burs, providing enamel cutting for cavity access or delicate caries excavation in the dentin layer.

Interactive visualization and tactile sensation from VR technology have opened new paradigms in clinical practice.[12] VR is a human-computer interface that facilitates highly interactive visualization and control of computer-generated, 3D-specific tissue models and their related components with sufficient detail and speed to simulate a sensorial experience similar to reality. The haptic systems provide force feedback through the tactile receptors in the skin and the proprioceptive receptors in muscles, tendons, and joints of the operator's hands.[13] In a caries-removal procedure, tactile sensation in the fingers is essential because touching the surface can be as useful as seeing it. Force feedback in VR simulators reveals the advantage in calculus and caries detection.[16,32] A multilayered virtual tooth model was generated to provide different tactile sensations in each layer of tooth structure that simulated the real experience in a haptic VR training system; thus, the participant felt a cutting perception similar to that of a real tooth by differential force feedback.

The results of a randomized controlled trial in this study showed the improvement in caries removal

outcomes for sixth-year dental students before and after participating in a designed training protocol. As in previous studies on clinical skill training,[24,25] the participants showed that performance improvement in scores of minimally invasive caries removal was not different after training sessions with the VR simulator and the extracted teeth in a phantom head. The explanation for these results is that the VR simulator is designed specifically to mimic the same hand motions and visualization as those used during dental procedures. Our model uses the virtual 3D model of carious teeth reconstructed from micro-CT images similar to those of Yoshida and others,[18] yet provides more detail in the infected and affected carious layers with different colors and hardnesses, which are helpful for visuo-tactile sensation. The students experienced caries removal in the same way as using a real tooth.

Interesting results were obtained regarding the caries removal in dentin. Students trained with the VR simulator had a better caries-removal performance score on the superficial and deep dentin layers, possibly due to the current VR simulator that contributes to the training of minimally invasive removal of carious dentin. The students were able to receive different color and force feedback when cutting through enamel, infected dentin, affected dentin, and pulp. They also received augmented feedback on the percentage of sound tooth structure and carious dentin removed from each layer during the operation. Moreover, changing from a high- to a slow-speed bur for cutting enamel and carious dentin was allowed in the current VR simulation. Some educators are concerned that students who become skillful with simulators might not be able to transfer these skills to the clinic,[33] but our students' posttraining performance confirmed that the minimally invasive caries-removal skills acquired on a VR simulator actually improved on real teeth.

Although VR simulation may require an initial investment in terms of software, costs must be balanced against those of traditional training. Nevertheless, VR training is becoming an attractive option as it requires little running cost; once bought, these devices are always available for use and allow for repeatable skills training. The limitation of this work is that students interact with a 3D model of a single tooth that is reconstructed from micro-CT images. It would be useful to incorporate more virtual environments of the whole clinical setting by using a head-mounted display to get true 3D and, especially, hand-tool alignment. More work should be done to incorporate outcome scoring and video feedback to show where errors occur in the procedure.

CONCLUSIONS

The initial results from developing a multilayered caries model and visuo-tactile VR simulator for minimally invasive caries-removal training are very encouraging. Training on a VR simulator and conventional extracted teeth had equivalent effects in improving minimally invasive caries-removal performance. This simulator, with further refinement, has excellent potential to benefit the minimally invasive approach.

Acknowledgements

This study was supported by the National Research Council of Thailand and the Thailand Research Fund (RDG6050029) and partially supported through a fellowship from the Hanse-Wissenschaftskolleg Institute for Advanced Study, Delmenhorst, Germany to Haddawy and Suebnukarn.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Thammasat University. The approval code for this study is 103/2559.

Conflict of Interest

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

(Accepted 24 August 2017)

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Mechanical and Surface Properties of Monolithic Zirconia

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Clinical Relevance

Restorations produced with monolithic zirconia are frequently used to replace those made with conventional zirconia and veneering porcelain. However, for correct use, it is important to know key material features, such as mechanical strength and fractographic behavior.

SUMMARY

Purpose: This study compared monolithic zirconia with conventional ones based on mean roughness (Ra), Vickers hardness (VHN), topography, transmittance, grain size, flexural strength (FS), Weibull modulus, and fractographic behavior.

Methods and Materials: One monolithic (Prettau Zircon [PR group]) and two conventional (ICE Zirkon Transluzent [IZ group] and Bloom-Zir [BL group]) zirconias were used. Specimens were tested using a profilometer, a microhardness tester, a scanning electron microscope, a spectrophotometer, and a Universal Testing Machine (EMIC DL 2000). Ra, VHN, grain size, and transmittance were analyzed using the Kruskal-Wallis test associated with Dunn test (α=0.05). FS was analyzed using one-way analysis of variance with the Tukey honestly significant difference test (α=0.05).

Results: Means and standard deviations of roughness, after sintering (Ra, in μm) and VHN, were, respectively, 0.11 ± 0.01, 1452.16 ± 79.49, for the PR group; 0.12 ± 0.02, 1466.72 ± 91.76, for the IZ group; and 0.21 ± 0.08, 1516.06 ± 104.02, for the BL group. BL was statistically rougher ($p<0.01$) than PR and IZ. Hardness was statistically similar ($p=0.30$) for all groups. Means and standard deviations of FS (in MPa) were 846.65 ± 81.97 for the PR group, 808.88 ± 117.99 for the IZ group, and 771.81 ± 114.43 for the BL group, with no statistical difference ($p>0.05$). Weibull moduli were 12.47 for the PR group, 7.24 for the IZ group, and 6.31 for the BL group, with no statistical differences. The PR and BL groups had higher transmittance values and grain sizes than the IZ group ($p<0.05$). Although the BL group had some fractures that originated in the center of the tensile

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DOI: 10.2341/17-019-L

surface, fractographic analyses showed the same fracture pattern.

Conclusions: All tested zirconia showed similar VHN, and the monolithic zirconia had similar roughness compared to one of the conventional zirconias. In addition, the monolithic zirconia showed similar flexural strength and Weibull modulus compared to the others even though its mean grain size was larger. The total transmittance of monolithic zirconia was higher than only one of the conventional zirconias tested.

INTRODUCTION

More than a decade ago, yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) was introduced in dentistry as a framework material that, for esthetic reasons, needs to be veneered with glass-ceramic or feldspathic porcelain.[1-3] Since then, zirconia has been widely used in various clinical situations due to its high esthetic potential, high biocompatibility, good dimensional and chemical stability, and high fracture toughness when compared to other dental ceramics.[4-6]

However, some clinical problems emerged with the use of Y-TZP, as it requires being veneered due to its high opacity.[7,8] Unfortunately, the veneering layers are prone to fracture[9-11] and have been associated with delamination and chipping.[2,7,12-16] Apart from significant improvements related to heating and cooling rates during porcelain sintering,[1,7,17,18] better framework designs,[7,19] and higher uniformity of the veneering layer (thickness),[7,20] Pjetursson and others[21] reported that 15% of Y-TZP restoration replacement occurred due to delamination and 20% due to chipping after five years of clinical follow-up.

In order to eliminate the weak veneering layer, monolithic zirconia restorations with higher translucency were developed by means of adding different dopants, coloring liquids, and changing the sintering temperatures.[7,8] Monolithic zirconia is a unique ceramic system with multiple clinical applications, including those with high esthetic demands,[22] and it is more easily processed than bilayered ones, having lower final cost.

Monolithic restorations are manufactured using computer-aided design/computer-aided manufacturing technology. Since these restorations are not veneered with porcelain, they can be finished by means of either polishing or applying a glaze layer.[2,19] In comparison to veneered restorations, full-contour zirconia restorations have the clinical advantage of allowing production of prostheses with significantly reduced thickness (only 0.5 mm for posterior restorations).[19,23-27]

Multiunit, monolithic prostheses need further investigation to determine if they can withstand as much chewing force as prostheses using regular Y-TZP as their framework. In this regard, determination of the flexural strength of ceramic materials can be helpful, especially after verifying whether the obtained strength meets strength standards, such as ISO 6872.[28] Flexural strength data of ceramic materials should preferably be analyzed using Weibull statistics, which describes the asymmetrical strength distribution resulting from the flaw population in the material microstructure.[29-32] In this analysis, the most commonly used parameter is the Weibull modulus (m), which is a measure for the scatter of strength data.[32,33] In addition to obtaining flexural strength data, it is important to carry out a descriptive fractographic analysis, which helps identify the failure origin and provides information about the loading conditions.[34]

Although monolithic zirconia was developed to overcome the limitations of conventional zirconia, comparisons between these two materials regarding their mechanical and optical properties are still scarce. Density, porosity, grain size, and the chemical nature of the material influence not only the optical but also the mechanical properties.[6,8,12,35,36] Thus, the aim of this study was to compare one monolithic zirconia material with two conventional zirconia materials in terms of their mean roughness (Ra), Vickers hardness (VHN), topography, transmittance, flexural strength, Weibull modulus, and fracture mode. The null hypothesis was that there would be no difference among the monolithic and conventional materials for any of the properties evaluated.

METHODS AND MATERIALS

Three commercially available zirconia ceramics were used: Prettau Zircon (PR group, n=15; Zirkonzahn GmbH, Gais, Italy), ICE Zirkon Transluzent (IZ group, reference group, n=15; Zirkonzahn), and BloomZir (BL group, n=15; Bloonden, Bioceramics Co, Hunan, China). PR is monolithic zirconia; IZ and BL are conventional zirconia. IZ and PR are different zirconias from the same manufacturer, and BL is relatively new on the market and, according to the manufacturer, is a high-translucency conventional zirconia. Sample size was calculated

after a pilot study considering $\beta = 0.80$ and $\alpha = 0.05$ for all tests.

Bar-shaped specimens (25×5×1.5 mm) were cut from presintered blocks using a high-precision sectioning saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) with a low-speed diamond disk (Series 15LC Diamond, Buehler) under water cooling. A calibrated operator manually polished the bars on all sides using #1200, #1500, and #2000 SiC papers (401Q, 3M, Sumaré, Brazil). A chamfer on the edges was made using rubber tips (126c, Edenta, Labordental, São Paulo, Brazil) according to ISO 6872.[28]

The bars were sintered in a furnace (Zirkonofen 600/V2, Zirkonzahn) following the manufacturer's instructions. The IZ and BL groups were sintered for eight hours at 1500°C, and the PR group was sintered for 8.2 hours at 1600°C.

Mean roughness (Ra, μm) values were determined for all specimens with an accuracy of 0.01 μm using a profilometer (Mitutoyo SJ 400, Mitutoyo Corp., Yokohama, Japan) with length of 2.5 mm, active tip radius of 5 μm, and speed of 0.5 mm/s at three different locations on each side of the specimen. The measurement locations were the same for all specimens, one in the center and the others equidistant (~5 mm) from the center, resulting in six measurements per specimen. The roughness measurements were made before ($Ra_{presintered}$) and after ($Ra_{sintered}$) the sintering process.

VHN was measured for all specimens using a microhardness tester (MMT-3, 1600-6300, Buehler) with a load of 500 gf applied for 30 seconds at four different regions to obtain an average for each bar.

Flexural strength (FS) was assessed for all specimens using a universal testing machine (EMIC DL 2000, Equipamentos e Sistemas de Ensaio Ltda, São José dos Pinhais, Brazil) with a four-point bending design (5 kN, 1 mm/min) in accordance with ISO 6872.[28] The FS values were calculated using the formula $\sigma = 3PL/4wb^2$, where σ = flexural strength in MPa, P = force in newtons at the moment of the fracture, L = the distance between the outer supports in millimeters, w = the width of the specimen in millimeters, and b = the thickness of the specimen in millimeters.

The reliability of the materials was calculated by the determination of Weibull modulus (m). The equation $P(\sigma) = 1 - \exp(-\sigma/\sigma_0)^m$ was applied to calculate the Weibull modulus, where $P(\sigma)$ is the fracture probability, σ is the fracture strength at a given $P(\sigma)$, σ_0 is the characteristic strength, and m is the Weibull modulus, which is the slope of the $1n$ ($1n$ $1/1 - P$) vs σ plots.[33,37-39]

For fractographic analysis, after the FS test, all specimens were cleaned in an ultrasonic bath using distilled water (five minutes) and isopropyl alcohol (five minutes), dried, and examined using a stereomicroscope (CCD, Olympus, Center Valley, PA, USA) in order to identify the fracture origin and to confirm whether the fracture started due to the flexural force. Magnifications ranged from 1× to 5×, and the illumination angle was changed many times to favor observation of crack features. A preliminary observation indicated areas of interest for further examination under scanning electron microscopy (SEM). To illustrate the microstructural features and analyze the fracture,[40] five representative specimens per group were cleaned again, dried, sputter coated with gold,[41] and observed under SEM (SM-300, Topcon, Tokyo, Japan) with magnifications from 300× to 4000×. Other micrographs (5000× and 10,000×) were performed for microstructural characterization of the surface specimens. For better identification of voids, a high-contrast and high-brightness image was made.

Transmittance measurements were made in five specimens per group using a spectrophotometer (CM 3700d, Konica Minolta, Singapore) in transmittance mode, with wavelengths ranging from 360 to 740 nm at intervals of 10 nm. The total transmittance (T) was calculated according to

$$T(\%) = (L_{specimen}/L_{source}) \times 100,$$

where L is the luminance of the specimen and of the source, respectively. L_{source} was obtained by making one measurement of L without any specimen placed in the optical path, resulting in an L_{source} value of 30,000. This value corresponded to 100% of transmittance and served as the baseline for calculations.

The Feret method was used to compare the zirconia grain sizes and the transmittance results.[42] The grains were measured in the 10,000× SEM micrographs (n=3) using Image J software (National Institutes of Health, Bethesda, MD, USA).

All data were submitted to the normality and homoscedasticity tests. Ra, VHN, and FS were analyzed using one-way analysis of variance (α=0.05) and the Tukey honestly significant difference test (α=0.05). The paired t-test (α=0.05) was used to compare the Ra before and after sintering. Grain size and transmittance data were analyzed

Table 1: *Means and Standard Deviations for Roughness ($Ra_{presintered}$ and $Ra_{sintered}$ in μm) and Vickers Hardness (VHN) According to the Experimental Groups*

Groups	$Ra_{presintered}$	$Ra_{sintered}$	VHN
PR	0.08 ± 0.01 Ba	0.11 ± 0.01 Ab	1452.16 ± 79.49 a
IZ	0.08 ± 0.01 Ba	0.12 ± 0.02 Ab	1466.72 ± 91.76 a
BL	0.07 ± 0.01 Ba	0.21 ± 0.08 Aa	1516.06 ± 104.02 a

Different lowercase letters indicate significant differences ($p<0.05$) among rows. Different uppercase letters indicate significant differences ($p<0.05$) among columns.

using the Kruskal-Wallis test associated with Dunn ($\alpha=0.05$).

RESULTS

The means and standard deviations for roughness ($Ra_{presintered}$ and $Ra_{sintered}$) and VHN are shown in Table 1. There was no significant difference among the roughness values before the sintering process ($p=0.10$); however, sintering increased roughness for all groups ($p<0.05$) with significant differences among groups ($p<0.01$). The BL group was rougher than the other groups, and PR and IZ showed statistically similar roughness. VHNs were statistically similar ($p=0.30$).

The means and standard deviations for FS (MPa), the Weibull (m) statistical analysis, and respective confidence intervals (95%) are shown in Table 2 and Figure 1. There was no statistical difference between groups ($p>0.05$) for FS as for the Weibull modulus (m) (all confidence intervals overlapped).

The first- and third-quartile percentages of spectral transmittance (T%), as well as the median, are shown in Table 3 and in Figure 2. The PR and BL groups had similar transmittance percentages, which were statistically higher than those obtained for IZ (Table 3).

The SEM micrographs to identify fracture origin are shown in Figures 3 and 4. The hackle lines, mirror, origin, and direction of crack propagation are highlighted in these figures. The greatest magnification images (from 300× to 4000×) were chosen to show the fracture origin; thus, the compression curl does not appear, but an asterisk (*) was placed on the top side of the specimen in the direction of the compression curl for better understanding. All specimens had the compression curl on the top side where the load was applied and the origin of the fracture at the bottom, being the tension force responsible for the fracture.

The majority of the specimens showed fracture origin on the tensile side of the specimen near its corner, and the direction of crack propagation went from corner to center and top of the specimen (Figure 3). The same fracture pattern was observed for all groups, although some fractures originated at the center of the tensile surface for the BL group, being the direction of crack propagation from center to sides and top of the specimen (Figure 4).

Figure 5 shows SEM images at 5000× magnification; it is possible to note that the grains of the PR (a) and BL (c) groups were larger than those of the IZ group (b). The surfaces of the IZ and PR groups were more homogeneous, with rounded grains, all at the same level related to the surface. On the other hand, the grains of the BL group were much more heterogeneous, with grains at different surface levels. Figure 6 shows a high-brightness and high-contrast image (10,000×) with identification of voids. It can be seen that IZ group presented less voids, while PR and BL showed similar quantities.

Figure 6a through 6c were used to apply the Feret method. Table 4 shows the median and the first and third quartile of grain size (in micrometers). PR and BL showed similar mean grain sizes, which were significantly larger than the mean size obtained for IZ.

DISCUSSION

This study aimed to compare the mean roughness, VHN, topography, transmittance, grain size, flexural

Table 2: *Mean Flexural Strength (FS [MPa]), Weibull Modulus (*m*), and Respective Confidence Intervals (CI = 95%)*

Groups	FS (MPa)	95% CI (FS)	*m*	95% CI (*m*)
PR	846.65 ± 84.85 a	806.48-890.39	12.47 a	8.30-18.72
IZ	808.88 ± 117.99 a	741.79-874.69	7.24 a	5.01-10.47
BL	771.81 ± 114.43 a	696.65-840.09	6.31 a	4.47-8.92

Different letters indicate statistically significant differences ($p<0.05$) among rows.

Table 3: *Total Transmittance Medians (T%) of Each Group*

Groups	T%
PR	25.02 (21.10, 27.01) a
IZ	21.46 (18.94, 23.09) b
BL	23.72 (21.39, 25.54) a

Values in parentheses are the first quartile and the third quartile, respectively. Different letters indicate statistically significant differences among rows.

strength, Weibull modulus, and fractographic behavior of three different zirconia ceramics to better understand the behavior of monolithic materials. The null hypothesis was partially rejected because there were significant differences for some properties of the zirconia evaluated.

Several studies have examined the mean roughness (Ra) of dental restorative materials[22,36,43-47] due to its importance in early biofilm interlocking and further maturation process[43] as well as its crucial role in the resistance of dental ceramics,[44-46] usually with a significant, negative correlation with FS.[26,39,48] Moreover, roughness is directly associated with the translucency of the material[22,36,47] since smooth surfaces could contribute to better esthetic performance, promoting less additional loss of incident light.[47]

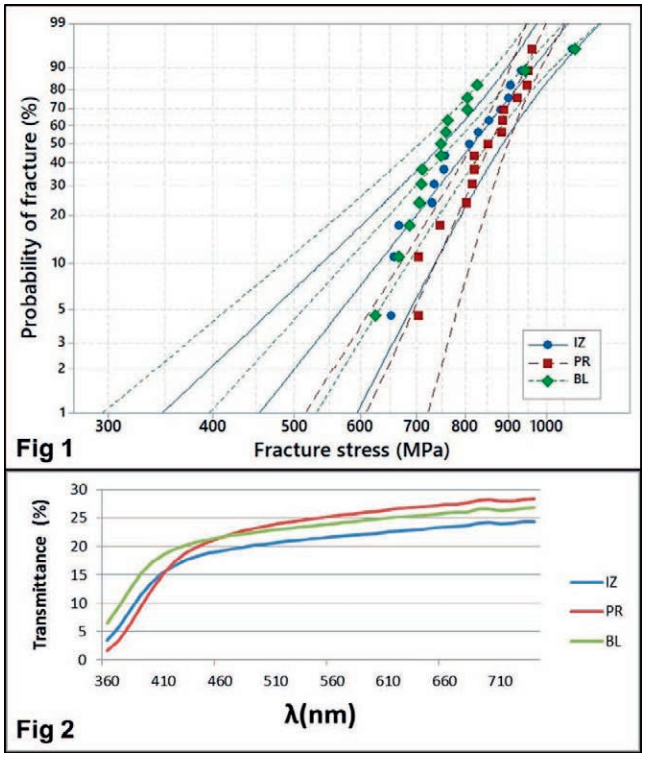

Figure 1. *Weibull plots of fracture data for PR, IZ, and BL groups.*
Figure 2. *Spectral transmittance of each experimental group.*

Fig 3

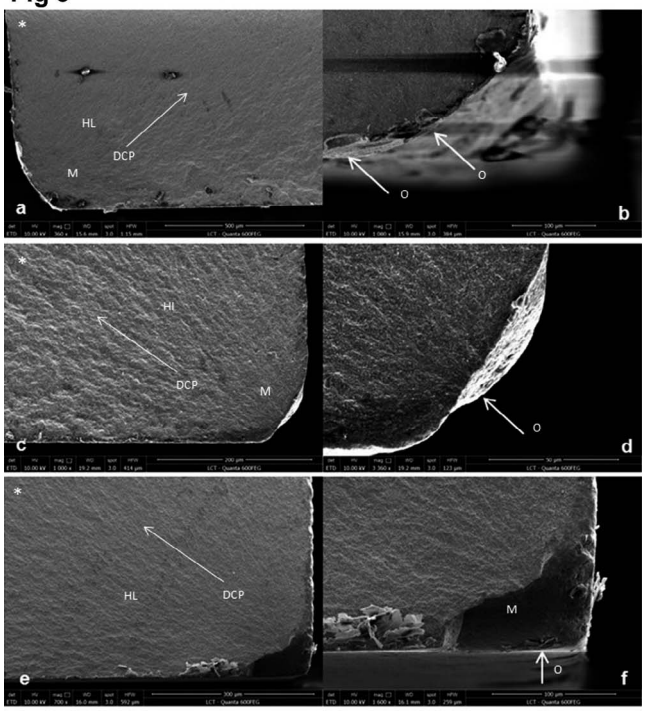

Fig 4

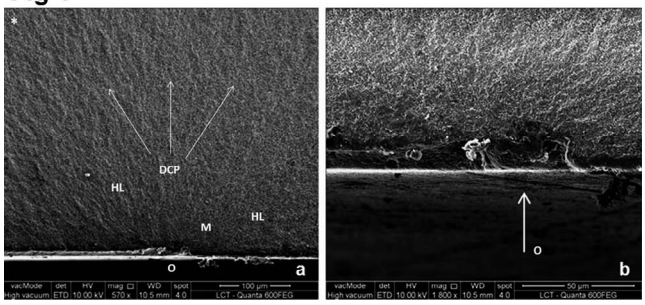

Figure 3. *Scanning electron micrographs: PR group (a and b), IZ group (c and d), and BL group (e and f), with different magnifications: (a) 360×, (b) 1080×, (c) 1000×, (d) 3360×, (e) 700×, and (f) 1600×. HL, hackle lines; DCP, direction of crack propagation; M, region of the mirror; O, origin of the fracture;*, top of the specimen.*
Figure 4. *Fractographic analysis of the BL group: (a) 570× and (b) 1800×. HL, hackle lines, DCP, direction of crack propagation; M, region of the mirror; O, origin of the fracture; *, top of the specimen.*

The Ra values obtained in this study are consistent with values obtained by other authors that ranged

Table 4: *Medians of Grain Sizes (μm)*

Groups	Grain Sizes
PR	0.79 (0.63, 0.95) a
IZ	0.48 (0.39, 0.59) b
BL	0.71 (0.60, 0.90) a

Values in parentheses are the first quartile and the third quartile, respectively. Different letters indicate statistically significant differences ($p<0.05$) among rows.

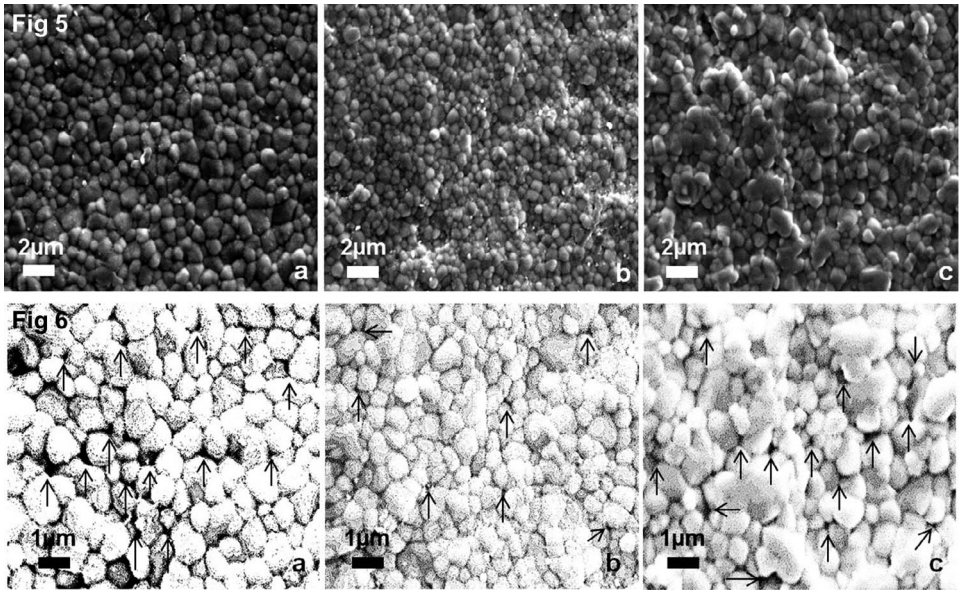

Figure 5. *Surface topography of the groups at 5000× magnification: (a) PR, (b) IZ, and (c) BL.*
Figure 6. *High brightness and contrast micrographs of the groups at 10,000× magnification: (a) PR, (b) IZ, and (c) BL. Arrows indicate voids.*

from 0.18 to 0.98 µm.[44,49-52] This wide range of values can be explained by variations in different surface treatments, polishing systems, zirconia grain sizes, pores, and flaw population.[44,51,53,54] After polishing and before sintering ($Ra_{presintered}$), the Ra was similar for all groups, indicating the standardization of polishing; however, after sintering ($Ra_{sintered}$), all groups had their roughness increased. A small increase in roughness can be expected due to surface modification, such as grain growth; however, for BL, this increase was higher, making it statistically different from the others. The Ra values obtained in this investigation can be considered clinically acceptable since only BL had Ra near 0.20 µm, a known threshold for plaque accumulation.[55] However, all groups showed Ra values above 0.5 µm, which is considered a Ra value detectable by the tongue.[56] The higher Ra measured for BL was explained by the SEM images (Figure 5c) since a more irregular surface and grains with asymmetrical size and form were observed for this material.

The VHN values found in this study for conventional and monolithic zirconia are consistent with values found in the literature, that is, around 1300 VHN,[35,57,58] with no statistical differences observed among the groups. The hardness of monolithic zirconia is important because the absence of veneering porcelain leaves the zirconia surface in direct contact with the antagonist tooth. A material with greater hardness may have greater mechanical strength, but it is difficult to determine whether this will result in higher wear rates for the antagonist tooth.[59] According to Goo and others,[60] the hardness of zirconia is twice that of dental porcelains. Due to this higher hardness, more enamel wear could be expected when monolithic zirconia is used. However, Stawarczyk and others[59] showed that the highest wear rate was observed for veneered and glazed zirconia as compared to polished monolithic zirconia. In the same study, the authors noted that monolithic zirconia resulted in higher rates of enamel cracks.

The means of FS obtained in the present study were either lower[24,61] or similar to those found in previous studies,[5,41] but these strength values are high enough to withstand the masticatory forces applied to three-unit fixed partial dentures.[28] Differences regarding FS obtained in different studies are usually related to the different methodological approaches and to the relationship between the strength of all-ceramic materials and the variation in the flaw population of different materials.[62]

The statistically similar FS results found for PR in comparison with the other two materials is not in agreement with the literature that showed lower values of FS for other monolithic zirconia.[63-65] In contrast, Flinn and others[61] used the Prettau zirconia and obtained higher values than those obtained in the present study of four-point FS test (1328±89.9 MPa). Muñoz and others[66] using a biaxial flexure test obtained statistically similar values between Prettau zirconia and Ice Zircon.

Some authors associate the increase in grain size with the decrease in the FS of the material;[63-65] therefore, it was expected that PR would have similar FS in comparison to BL. However, the lack of statistical difference among the FS values of all

material indicates that the increase in grain size did not affect the FS of the monolithic zirconia tested. The strength of zirconia specimens is associated with flaws such as porosity, agglomerates, inclusions, and large grains.[38] IZ showed less quantity of voids in the SEM (Figure 6); however, when these voids were statistically analyzed by the Weibull modulus, the materials had a statistically similar flaw population (Table 2). Therefore, the similarity among the FS values observed for these monolithic zirconias may be associated with other factors, such as amount of dopants, chemical composition, and crystalline structure. These variables need to be further investigated.

The Weibull modulus (m) of dental ceramics usually ranges from 5 to 15,[59,67] which is consistent with the present study. A higher m indicates a material that is more reliable under clinical conditions. This is because it has lower variation in flaw size in a certain volume of material, suggesting that the defects are uniform and evenly distributed throughout the entire volume.[30-32,68] Since the m values obtained in this investigation were similar for all materials, it is possible to infer that the flaw population was similar among the materials tested.[32,69]

These results could be corroborated by the fractographic analysis (Figures 3 and 4). Since advanced ceramics such as zirconia usually display linear stress-strain behavior, the lack of ductility, combined with the presence of flaws that have various sizes and orientations, leads to scatter in strength data.[38] Therefore, it is highly recommended that each failed test specimen be examined in order to identify the fracture origins.[38]

In the present study, the majority of the specimens showed surface flaws on the tensile side of the specimen, near the chamfer produced in the bend bar (Figure 3). BL also exhibited some fracture origins located at the center of the tensile surface (Figure 4). Fracture origins were identified by means of fractography principals proposed by Quinn[70] and by Scherrer and others.[40] Several fractographic features were identified, such as the compression curl, the hackle lines, the mirror, and then the origin. In Figures 3 and 4, it is possible to note hackle lines, the direction of crack propagation, the mirror, and the origin. Hackle lines are lines that clearly indicate the direction of crack propagation.[70] They commonly form when the crack moves rapidly.[40] The fracture mirror is a smoother region that surrounds the origin of the fracture.[70] In these specimens, the mirror is not so characteristic as described by Quinn[70] for glasses; in Figures 3 and 4, it can be subtly noted between the origin and hackle lines. All specimens had the compression curl on the top and the origin of the fracture on the bottom, indicating that the flexural test was carried out correctly and that the fractures were due to flexural force.

The PR and BL groups obtained higher transmittance compared to IZ. In the visible light region (wavelength from 360 to 740 nm), the median percentages of transmittance was 21.46% for IZ vs 25.02% for PR and 23.72% for BL. The higher transmittance of monolithic zirconia found in the present study is consistent with other studies that also showed higher translucency for these materials in comparison with traditional ones.[22,71] It is known that the translucency of dental ceramics is affected by various factors, such as grain size, pores, sintering temperature, and surface roughness.[22,36,71] Most studies attribute the increase in transmittance to grain sizes that are smaller than the wavelength of the incident light and therefore avoid the birefringence phenomenon, which is responsible for light scattering in Y-TZPs.[36,71-73] However, when the grains become larger than the wavelength of the incident light, light scattering becomes inversely proportional to grain size.[36,74] So there are two main methods to produce a more translucent zirconia, as one can either increase or decrease the grain size. Usually, when the choice is to decrease the grain size, the strength of the material is not affected. However, when the grain size is increased, transmittance increases, and strength decreases. In the present study, groups having higher transmittance were those having higher grain size, PR (0.79 μm) and BL (0.71 μm), vs IZ (0.48 μm). Apparently, for the manufacturers of these materials, the choice was to increase the grain size to increase the transmittance. In the present study, this increase in grain size did not decrease the FS.

In addition, Krell and others[47] showed that the grain boundary causes light scattering. In the present study, BL and PR presented larger grains and therefore fewer grain boundaries, thus decreasing light scattering and increasing the total transmittance. Harianawala and others[22] also attributed the difference in transmittance between conventional and monolithic zirconia to the smaller number of internal defects and pores in the latter, which also decreases light scattering.

The grain sizes measured for the IZ and PR groups are within the range reported in the literature.[71,75,76] However, the grain sizes obtained for the BL group were greater than the size proposed in the literature for conventional zirconia (0.5 μm). It is important to control the grain size for conventional zirconia

because larger grains can facilitate the *t-m* transformation and result in degradation of the material over the long term.[52,64,75-77] However, for monolithic zirconia, materials with larger grains showed degradation similar to that of conventional zirconia.[78]

The present study has limitations, such as the fact that static loading for FS does not reproduce intraoral loading conditions[41] and does not take into account the effects of design, variation in thickness of the framework, the nature of human occlusion, and the loading environment. Additional studies, with specimen geometries used in clinical applications, should be pursued in the future.

CONCLUSIONS

Based on the results of the present study, it was concluded that all zirconias tested showed similar VHN and that the monolithic zirconia had similar roughness compared to the conventional zirconia (IZ group). In addition, the monolithic zirconia showed similar flexural strength and Weibull modulus compared to the others, even though its mean grain size was larger. The total transmittance of monolithic zirconia was higher than only one of the conventional zirconias tested (IZ group).

Acknowledgements

The authors wish to thank PhD student Erick de Lima from the Department of Biomaterials and Oral Biology, São Paulo Dental School, Universidade de São Paulo, for his contribution to this study. The group also wishes to thank the dental laboratory Frezzara, Araraquara, Brazil, for the contribution of the materials to the study.

Conflict of Interest

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

(Accepted 2 October 2017)

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©Operative Dentistry, 2018, **43-3,** E129-E151

Compliance of Randomized Clinical Trials in Noncarious Cervical Lesions With the CONSORT Statement: A Systematic Review of Methodology

A Reis • JL de Geus • L Wambier • M Schroeder • AD Loguercio

Clinical Relevance

Systematic reviews are the top level of evidence, and the results may help clinical decisions that are needed to provide the best treatment for patients. In face of that, the adherence of randomized clinical trials evaluating adhesive systems should be improved.

SUMMARY

The literature was reviewed to evaluate the compliance of randomized clinical trials (RCTs) with the CONsolidated Standards of Reporting Trials (CONSORT) and the risk of bias of these studies through the Cochrane Collaboration risk of bias tool (CCRT). RCTs were searched at Cochrane Library, PubMed, and other electronic databases to find studies about adhesive systems for cervical lesions. The compliance of the articles with CONSORT was evaluated using the following scale: 0 = no description, 1 = poor description, and 2 = adequate description. Descriptive analyses about the number of studies by journal, follow-up period, country, and quality assessments were performed with CCRT for assessing risk of bias in RCTs. One hundred thirty-eight RCTs were left for assessment. More than 30% of the studies received scores of 0 or 1. Flow chart, effect size, allocation concealment, and sample size were more critical items, with 80% receiving a score of 0. The overall CONSORT score for the included

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DOI: 10.2341/17-060-L

studies was 15.0 ± 4.8 points, which represents 46.9% of the maximum CONSORT score. A significant difference among countries was observed ($p<0.001$), as well as range of year ($p<0.001$). Only 4.3% of the studies were judged as at low risk; 36.2% were classified as having unclear risk and 59.4% as having high risk of bias. The adherence of RCTs evaluating adhesive systems to the CONSORT is low with unclear/high risk of bias.

INTRODUCTION

Due to the development of adhesive systems, macro-mechanical retention is no longer essential. The use of adhesive systems allows good retention of restorative materials without the need for macro-mechanical retention. This might explain the rapid evolution and release of several commercial adhesive formulations. Etch-and-rinse adhesives, which require preliminary removal of the smear layer, are offered in two and three steps. Self-etch adhesives, capable of simultaneously demineralizing and infiltrating the dental substrates, are sold in one or two clinical steps. More recent and versatile systems, named as universal systems, can either be used in an etch-and-rinse or self-etch mode.

Despite the benefits that adhesive systems have made possible, clinicians are exposed to adhesives that use different bonding strategies with different levels of simplification. To make things more complicated, for each one of these combinations, a high number of commercial brands are available.

Laboratory testing is a very useful method for comparing the bonding performance of adhesive systems, but thus far, authors of few studies have found any correlation of their results with clinically important outcomes. On the other hand, clinical trials can provide reliable and direct evidence to guide clinicians to choose dental materials. The comparison of bonding techniques and adhesive systems is usually performed with noncarious cervical lesions (NCCLs), as these lesions lack macro-mechanical retention and therefore restoration loss is due to ineffective bonding, which is an objective and clinically important outcome for adhesive efficacy.

Randomized controlled trials (RCTs) represent the standard design for evaluation of health care interventions. Well-designed RCTs and systematic reviews of well-designed RCTs are on the top of the hierarchy of the levels of evidence. However, RCTs can yield biased results if they lack methodologic rigor.[1] Problems with the design and execution of RCTs raise questions about the validity and reliability of their findings that can end up with an underestimation or overestimation of the true intervention effect.[2-4]

In this way, one should appraise the quality of RCTs before any clinical decision making. This assessment depends on a good reporting/writing of the methods and results sections of the RCTs. In an attempt to standardize the reporting, a group of experts joined together in 1996 and produced the CONSORT statement,[5] which is a checklist with recommendations for reporting of clinical trials in biomedical literature. This CONSORT statement was revised in 2001,[6] and the most recent one was published in 2010.[7,8]

The compliance of RCTs with the CONSORT statement[7,8] was evaluated in several specialties of medicine,[9,10] as well as in some areas of dentistry, such as implantology, prosthodontics,[11,12] periodontology,[13] orthodontics,[14-16] and pediatric dentistry.[17] Given the importance of RCTs in NCCLs for decision making during restorative procedures, the aim of this study was to systematically review the literature in peer-reviewed journals to evaluate 1) the compliance of recent RCTs with the CONSORT statement and 2) the risk of bias of these studies through the Cochrane Collaboration risk of bias tool (CCRT).

METHODS AND MATERIALS

This study was not registered *a priori* as no known register currently accepts protocols for methodology of systematic reviews.

Search Methods

The following electronic databases were used to identify eligible studies: Cochrane Library, MEDLINE via PubMed, EMBASE, Latin American and Caribbean Health Sciences Literature (LILACS) database, and the Brazilian Library in Dentistry (BBO). Citation databases such as Scopus and Web of Science (Table 1) were also searched. Additionally, the reference lists of all primary studies were searched for additional relevant publications, as well as the first page of the related articles' links to each primary study in the PubMed database. Articles in Japanese, Chinese, Arabian, and other Eastern languages were not included due to difficulties in the translation process.

The search strategy was first prepared for the MEDLINE database by using controlled vocabulary (MeSH terms) and free keywords. Then, the search

Table 1: *Search Strategy (16/04/16)*

Pubmed			
#1 tootherosion[MeSHTerms] ORtoothabrasion[MeSHTerms] ORtoothcervix[MeSHTerms] OR "cervicallesion"[Title/Abstract] OR "cervicallesions"[Title/Abstract] OR "classV"[Title/Abstract] OR "class 5"[Title/Abstract] ORabfraction[Title/Abstract] OR "toothcervix"[Title/Abstract] OR "tootherosion"[Title/Abstract] OR "toothabrasion"[Title/Abstract]	#2 dentin-bondingagents[MeSHTerms] OR "adhesivesystem"[Title/Abstract] OR "adhesivesystems"[Title/Abstract] OR "bondingagent"[Title/Abstract] OR "bondingagents"[Title/Abstract] OR "dentaladhesive"[Title/Abstract] OR "dentaladhesives"[Title/Abstract] OR "adhesivematerial"[Title/Abstract] OR "adhesivematerials"[Title/Abstract] OR "etch-and-rinse"[Title/Abstract] OR "total-etch"[Title/Abstract] OR "self-etch"[Title/Abstract] OR "self-etching"[Title/Abstract] OR "all-in-one"[Title/Abstract] OR "one-bottle"[Title/Abstract]	#3 compositeresins[MeSHTerms] ORdentalrestoration, permanent[MeSHTerms]OR "resincomposite"[Title/Abstract] OR "resincomposites"[Title/Abstract] OR "compositeresin"[Title/Abstract] OR "compositeresins"[Title/Abstract] OR "resinrestoration"[Title/Abstract] OR "resinrestorations"[Title/Abstract] OR "compositerestoration"[Title/Abstract] OR "compositerestorations"[Title/Abstract]	#4 (randomizedcontrolledtrial[pt] ORcontrolledclinicaltrial[pt] ORrandomizedcontrolledtrials[mh] ORrandomallocation[mh] ORdouble-blindmethod[mh] ORsingle-blindmethod[mh] ORclinicaltrial[pt] ORclinicaltrials[mh] OR ("clinicaltrial"[tw]) OR ((singl*[tw] ORdoubl*[tw] ORtrebl*[tw] ORtripl*[tw]) AND (mask*[tw] ORblind*[tw])) OR (placebos[mh] ORplacebo*[tw] ORrandom*[tw] ORresearchdesign[mh:noexp] ORcomparativestudy[pt] ORevaluationstudiesastopic[mh] ORfollow-upstudies[mh] ORprospectivestudies[mh] ORcontrol*[tw] ORprospective*[tw] ORvolunteer*[tw]) NOT (animals[mh] NOThumans[mh]))
#1 AND #2 AND #3 AND #4			
Scopus			
#1 (TITLE-ABS-KEY (**"tooth erosion"**) OR TITLE-ABS-KEY (**"tooth abrasion"**) OR TITLE-ABS-KEY (**"tooth cervix"**) OR TITLE-ABS-KEY (**"cervical lesion"**) OR TITLE-ABS-KEY (**"class V"**) OR TITLE-ABS-KEY (**"class 5"**) OR TITLE-ABS-KEY (**abfraction**))	**#2**TITLE-ABS-KEY("adhesive system") OR TITLE-ABS-KEY("bonding agent") OR TITLE-ABS-KEY("dental adhesive") OR TITLE-ABS-KEY("adhesive material") OR TITLE-ABS-KEY("etch-and-rinse") OR TITLE-ABS-KEY("total-etch") OR TITLE-ABS-KEY("self-etch*") OR TITLE-ABS-KEY("all-in-one") OR TITLE-ABS-KEY("one-bottle")	**#3**TITLE-ABS-KEY("composite resin") OR TITLE-ABS-KEY("resin composite") OR TITLE-ABS-KEY("resin restoration") OR TITLE-ABS-KEY("composite restoration")OR TITLE-ABS-KEY (**"dental restoration"**) AND (LIMIT-TO (SUBJAREA , **"DENT"**))
#1 AND #2 AND #3			
Web of Science			
#1 Topic: ("tooth erosion") *OR*Topic: ("tooth abrasion") *OR*Topic: ("tooth cervix") *OR*Topic: ("cervical lesion*") *OR*Topic: ("class V") *OR*Topic: ("class 5") *OR*Topic: (abfraction)	**#2**Topic: ("adhesive system*") OR Topic: ("bonding agent*") OR Topic: ("dental adhesive*") OR Topic: ("dentin bonding") OR Topic: ("adhesive material*") OR Topic: ("etch and rinse") OR Topic: ("total etch ") OR Topic: ("self etch*") OR Topic: ("all in one ") OR Topic: ("one bottle ")	**#3**Topic: ("resin composite*") *OR* Topic: ("dental restoration*")OR Topic: ("composite resin*") OR Topic: ("resin restoration*") OR Topic: ("composite restoration*")	
#1 AND #2 AND #3			
Lilacs and BBO			
#1 (MH:"tooth erosion" OR MH:"tooth abrasion" OR MH:"tooth cervix" OR "cervical lesion" OR "lesão cervical" OR "lesión cervical" OR "cervical lesions" OR "lesões cervicais" OR "lesiones cervicales" OR "class V" OR "classe V" OR "clase V" OR"class 5" OR "classe 5" OR "clase 5" OR abfraction OR "abfração" OR "abfracción")	**#2**(MH:"dentin-bonding agents" OR "adhesive system" OR "adhesive systems" OR "sistema adesivo" OR "sistemas adesivos" OR "sistema adhesivo" OR "sistemas adhesivos" OR "bonding agent" OR "bonding agents" OR "agentes de união" OR "agentes de unión" OR "agentes de ligación" OR "agentes de enlace" OR "dental adhesive" OR "dental adhesives" OR "adesivo dental" OR "adhesivo dental" OR "adesivos dentais" OR "adhesivos dentales" OR "adhesive material" OR "material adesivo" OR "material adhesivo" OR "adhesive materials" OR "materiais adesivos" OR "materiales adhesivos" OR "adesivo dentinário" OR "adesivos dentinários" OR "adhesives dentinarios" OR "adhesive material" OR "adhesive materials" OR "dentin bonding agent" OR "dentin bonding agents" OR "etch-and-rinse adhesive" OR "etch-and-rinse adhesives" OR "adesivo convencional" OR "adesivos convencionais" OR "adhesive convencional" OR "adhesives convencionales" OR "total-etch adhesive" OR "total-etch adhesives" OR "condicionamento ácido total" OR "adhesivo de grabado total" OR "adhesivos de grabado total" OR "self-etch adhesive" OR "self-etch adhesives" OR "adesivo autocondicionante" OR "adesivos autocondicionantes" OR "adhesive autograbado" OR "adhesives autograbados" OR "self-etching adhesive" OR "self-etching adhesives" OR "all-in-one adhesive" OR "all-in-one adhesives" OR "adesivo de passo único" OR "adesivos de passo único" OR "adhesivo de paso unico" OR "adhesivos de passo unico" OR "one-bottle adhesive" OR "one-bottle adhesives" OR "adesivo de frasco único" OR "adesivos de frasco único")	**#3**(MH: "composite resins" OR MH: "dental restoration, permanent" OR "resin composite" OR "resin composites" OR "resina composta" OR "resinas compostas" OR "resina compuesta" OR "resinas compuestas" OR "composite resin" OR "composite resins" OR "compósito" OR "compósitos" OR "resin restoration" OR "resin restorations" OR "restauração de resina" OR "restauração de resinas" OR "restauración de resina" OR "restauraciones de resina" OR "composite restoration" OR "composite restorations"

Table 1: *Search Strategy (16/04/16) (cont.)*

		OR "restauração de compósito" OR "restaurações de compósitos" OR "restauração de resina composta" OR "restaurações de resinas compostas")
#1 AND #2 AND #3		
Cochrane Library		
#1 MeSH descriptor: [Tooth Erosion] explode all trees #2 MeSH descriptor: [Tooth Abrasion] explode all trees #3 MeSH descriptor: [Tooth Cervix] explode all trees #4 cervical next lesion?:ti,ab,kw #5 "class V":ti,ab,kw #5 "class 5":ti,ab,kw #7 abfraction:ti,ab,kw #8 tooth next cervix:ti,ab,kw #9 tooth next erosion:ti,ab,kw # 10 tooth next abrasion:ti,ab,kw #11 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10	#12 MeSH descriptor: [Dentin-Bonding Agents] #13 adhesive next system*:ti,ab,kw #14 bonding next agent*:ti,ab,kw #15 dental next adhesive*:ti,ab,kw #16 "dentin bonding agent":ti,ab,kw #17 "dentin bonding agents":ti,ab,kw #18 adhesive next material*:ti,ab,kw #19 "etch and rinse":ti,ab,kw #20 total next etch*:ti,ab,kw #21 "self etch*":ti,ab,kw	#22 "all in one ":ti,ab,kw #23 "one bottle":ti,ab,kw #24 #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 #25 MeSH descriptor: [Composite Resins] #26 MeSH descriptor: [Dental Restoration, Permanent] #27 resin next composite*:ti,ab,kw #28 composite next resin* #29 resin next restoration* #30 composite next restoration*:ti,ab,kw #31 #25 or #26 or #27 or #28 or #29 or #30 #32 #11 and #24 and #31

strategy was adapted to the other electronic and citation databases (Table 1). Only studies published in 1996 or later were included. This time period was chosen because the CONSORT statement was first published in 1996, and hence it would be unfair to expect that RCTs prior to this year would adhere to a standard that did not exist at the time of writing. Gray literature was not addressed because the study objective was to evaluate studies published in peer-reviewed journals.

Eligibility Criteria

Parallel and split-mouth RCTs that evaluated the performance of adhesive systems, restorative materials, or restorative and technique protocols in NCCLs of adult patients of any age group were included. RCTs should have at least two comparable groups, in which one of the groups was testing an adhesive system.

Articles could be excluded 1) if a clinical study did not perform a clinical evaluation, but rather was a laboratory evaluation; 2) if a study evaluated techniques for management of dentin hypersensitivity; 3) if there were conference abstracts, theses, or reports published in any media different from peer-reviewed journals; and 4) if studies were published earlier than 1996.

Initially, the articles were selected by title, and abstracts and duplicates were removed. Full-text articles were obtained, and subsequently, three reviewers (J.G., L.W., and A.R.) classified those that met the inclusion criteria.

Adherence to CONSORT Statement

An evaluation tool based on the items related to the methods and results from the 2010 CONSORT statement was developed[7,8] to evaluate the reporting completeness of RCTs (Table 2). A total of 12 items of the CONSORT were included in this CONSORT evaluation tool. As some of these items were subdivided, a total of 16 items were evaluated. The given score per item ranged from 0 to 2. In other words, 0 = no description, 1 = poor description, and 2 = adequate description. More details about the scoring process are found in Table 2. Each item was given equal weighting.

Before evaluation, the instrument was discussed between two experienced authors in clinical trials (A.D.L. and A.R.), pilot tested in 20 articles, and checked for accuracy and reproducibility by two evaluators. This process yielded modification of the instrument tool, as new possibilities for each score were observed and discussed during pilot testing.

Table 2: *Instrument Tool Developed From the 2010 CONSORT Statement to Evaluate the Compliance of the Studies With the CONSORT Statement*

CONSORT Item	Subitem	Score	Description
Trial design		2	The trial design is clearly written in the text (split mouth, cross-over, multiple restorations per patient, factorial, or cluster).
		0	The information is not reported.
		1	1. Information can be obtained by reading the manuscript, although the authors do not explicitly report it. 2. Consistence is lacking between sections of an article (e.g., abstract does not match the material and methods section; the presentation of the results does not match the description of the trial design; flow diagram presents different information, etc.).
Participants	Eligibility criteria	2	The inclusion and exclusion criteria is clear so that readers can know exactly to which population the data can be extrapolated.
		0	The information is not reported.
		1	1. Incomplete information of eligibility criteria compared to most of the studies in the field. 2. Presence of inconsistencies in the inclusion/exclusion criteria that prevent readers from knowing for which populations the intervention/control groups were performed.
	Settings and location	2	Clear description of the setting (academic, practice-based research, university, private clinics, etc.) and the date when the intervention was implemented.
		0	The setting and/or the location are not reported in the text.
		1	1. Authors describe either the setting or the date but never both. 2. This information can be obtained indirectly in the text.
Interventions		2	The interventions for each group are described with sufficient details to allow replication, including how they were actually administered.
		0	No description is given.
		1	Information is missing that prevents the replication of the interventions/comparators.
Outcomes		2	At least the primary outcomes were defined in detail, including how and when they were assessed. Considered as clear when the details are clear, but the authors did not use the term “primary outcome” or related synonyms.
		0	No definition of the primary outcome and/or secondary outcomes is given.
		1	1. The authors only report they have used specific criteria without detailing the most important outcomes of such criteria. 2. The description of the primary outcome and/or secondary outcomes is very superficial and does not allow replication of the method.
Sample size		2	Method of sample size calculation is described in a way that allows replication. The primary outcome for each sample size calculated should be identified. Elements of the sample size calculation for superiority trials are (1) the estimated outcomes in each group (which implies the clinically important target difference between the intervention groups); (2) the α (type I) error level; (3) the statistical power (or the "β [type II] error level); (4), for continuous outcomes, the standard deviation of the measurements should be reported. For equivalence trials, the equivalence limit instead of the effect size should be reported.
		0	No description is given in the article.
		1	The sample size is described but some parameters are missing so that it prevents replication.
Randomization	Sequence generation	2	1. Clear description of the random sequence generation. 2. Or clear description of a non-random sequence method.
		0	No information is given in the text.
		1	The authors only provide a very superficial description (such as the “groups were randomly allocated“) or do not provide sufficient information to allow replication of the randomization process.
	Allocation concealment	2	Clear description of the allocation concealment. See the Cochrane Collaboration tool for evaluation of the risk of bias.
		0	No information is given in the text.
		1	Partial reporting that prevents readers from fully replicating the method.

Table 2: *Instrument Tool Developed From the 2010 CONSORT Statement to Evaluate the Compliance of the Studies With the CONSORT Statement (cont.)*

CONSORT Item	Subitem	Score	Description
Blinding		2	1. The authors describe who is blinded in the study. 2. In single-blind studies (when this is clearly reported by the authors), just the description of participant or evaluator (the one blinded) is enough; however, when a study is double blind or triple blind all blinded people should be described. 3. The study describes just the participant or examiner blinded but one of these participants cannot be blinded by intrinsic features of the study design.
		0	No description of the blinding is given.
		1	Insufficient/partial information. For instance, (1) the authors describe examiners' blinding or participants' blinding, but never both. (2) The authors describe the study was blind or double-blind but do not specify who was blinded.
Statistical methods	Hypothesis testing	2	Statistical methods are described with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. Additionally, statistical tests employed by the authors seem to be adequate for the type of trial design and nature of the data collected.
		0	Statistical methods are not described.
		1	1. Not enough information is given to evaluate the statistical method used by the authors, and/or the type of statistical tests employed by the authors are inadequate for the trial design and/or nature of the data (e.g., tests that do not take into account the paired nature of the data when this is the case). 2. The authors describe several statistical tests but do not specify for each outcome they were applied.
	Estimated effect size	2	Authors report (at least for the primary outcome) the effect size and its precision (such as 95% confidence interval). Odds ratio, risk ratio, risk difference, mean difference, etc. are given.
		0	No descriptions of the effect size and 95% confidence interval are given.
		1	Information is incomplete.
Participant flow	Flow diagram	2	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome is described in the flow chart CONSORT diagram.
		0	The flow-chart is not presented in the article.
		1	1. Inconsistencies exist between the numbers described in the flow chart and other parts of the manuscript. 2. Incomplete diagram with missing information.
	Losses and Exclusions	2	1. For each group, losses and exclusions after randomization are described with reasons. 2. During reading, a reviewer can observe that no losses occurred to follow-up.
		0	1. No description of losses and exclusions is given.
		1	Incomplete information. For instance, 1. the authors describe the overall percentage of losses but this information is not specified per group, or 2. the authors describe the losses and exclusions but do not specify the reasons.
Baseline data		2	A table/text description containing baseline demographic and clinical characteristics of each group are presented in the article.
		0	No table/text description with baseline data or description is given in the body of the text.
		1	1. A table/ text description with baseline data is presented but the data is not distributed between the study groups and/or it is given in percentages instead of raw numbers. 2. Insufficient information about participants/lesions is provided. 3. Inconsistencies in the data presented can be observed.
Numbers analyzed		2	For each group and for each outcome, the number or participants (denominator) included in the analysis is clear.
		0	Authors do not report the numbers analyzed.
		1	No clear description of the number of participants (denominator) is included in the analysis of at least one of the outcomes. 2. Instead of reporting the raw number of participants, the authors report their data in percentages. 3. The authors fail to report the baseline number of patients included in each analysis. 4. Data can be obtained indirectly in the study.
Registration and protocol		2	The study was registered in a trial registry and the protocol number is provided.
		0	1. This information is not available in the manuscript. 2. Registration with an ethics committee is valid as trial registry
		1	The authors describe that the study was registered but do not provide the registration number and/or the number provided does not link to the study.

A single author (A.R.) performed the round of scoring using the CONSORT evaluation tool (Table 2), and only in case of doubt, a second author (A.D.L.) was contacted for discussion and final decision. Evaluators were not blinded to the study authors. This would not be possible as authors were familiar with the studies and could guess the researcher center by reading the paper.

Scoring System and Statistical Analysis

Descriptive analyses about the number of studies by journal, follow-up period, and country were described. Compliance with individual items of the CONSORT statement was analyzed to determine what clinical researchers should improve in their description. To do this, the percentage of studies per score in each item was provided in a chart.

To achieve an overall compliance score per article, the scores of the 16 items were summed. A trial with complete adequate descriptions (score 2) in all CONSORT items would receive a maximum score of 32. An average score was calculated by period of time, journal, and country. Comparison within each factor was performed with the Kruskall-Wallis and Mann-Whitney tests at a level of confidence of 95%. Linear correlation analysis between 2015 International Scientific Index (ISI) journal impact factor and the average CONSORT score was also performed.

Risk of Bias in Individual Studies

Quality assessments were performed by two independent reviewers, using the Cochrane Collaboration's tool for assessing risk of bias in RCTs.[18] The assessment criteria contained six domains: sequence generation, allocation concealment, blinding of the outcome assessors, incomplete outcome data, selective outcome reporting, and other possible sources of bias.

For each aspect of the quality assessment, the risk of bias was scored following recommendations of the *Cochrane Handbook for Systematic Reviews of Interventions 5.1.0* (http://handbook.cochrane.org). At the study level, the study was considered at low risk of bias if all domains received the same judgment. If at least one domain was judged as at unclear risk, the study was considered as having unclear risk of bias. On the other hand, if at least one domain was judged at high risk of bias, then the study was also at high risk of bias. During data selection and quality assessment, disagreements between reviewers were solved through discussion.

RESULTS

Characteristics of the Included Studies

From a total of 2191 screened articles, 2031 were excluded for not meeting the inclusion criteria. The full texts of 160 papers were obtained and assessed, and 22 papers were excluded for the following reasons: 1) 10 studies were not RCTs; 2) four studies compared only glass ionomer cements; 3) two studies were duplicates; 4) one study performed replica rather than clinical evaluation; 5) one study was an abstract; 6) one study was in the Chinese language; 7) one study was performed *in vitro*; 8) one study was performed in class I and II restorations; and 9) one study evaluated only desensitizers. After these exclusions, 138 RCTs were left for final assessment (Figure 1).

The included RCTs investigated several issues. Study authors usually compared 1) patient-related (eg, dentin sclerosis) and operator-related factors (eg, clinical experience); 2) different adhesive systems for bonding and desensitization; 3) different restorative materials; 4) curing methods, and 5) composite-resin-based vs glass ionomer and/or resin-modified glass ionomer cements. In some studies, more than one of these variables were evaluated.

Table 3 displays the 138 RCTs tabulated by their collected characteristics. The journals contributing with the most RCTs were *Operative Dentistry* (17.4%), followed by *American Journal of Dentistry* (12.3%), *Clinical Oral Investigations* (10.1%), and *Journal of Dentistry* (10.1%). Approximately 26.9% of the publications were published in 16 different journals. The countries with most publications were Brazil (31.2%) and the United States (18.1%), representing together approximately 50% of all publications in the field. An increase in the number of articles is occurring over time, but unfortunately, more than half (62.3%) of the publications are of short-term duration (6 months to 2 years).

Study Compliance With Each of the CONSORT Instrument Tool Items

Figure 2 displays the percentage of studies in each item of the CONSORT Statement. Regarding the item numbers analyzed, losses/exclusions, eligibility criteria, and intervention, approximately 70% of the studies were scored as 2, meaning adequate reporting of these items.

In all other items, more than 30% of the studies received a score of 1 (poor reporting) or a score of 0

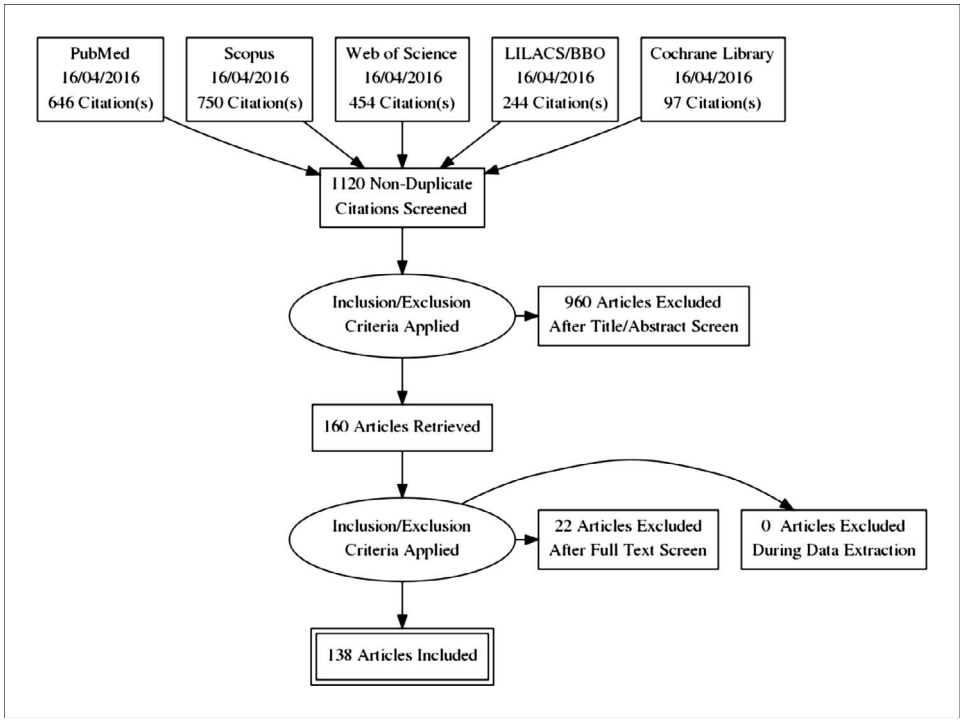

Figure 1. *PRISMA flowchart diagram showing the number of articles in the different phases of the study.*

(no report). This was more critical in the item's protocol, flow chart, effect size, allocation concealment, and sample size, where more than 80% of the studies were scored as 0 (no report).

Average CONSORT Score per Study Characteristics

The overall CONSORT score for the included studies in this review was 15.0 ± 4.8 points, which

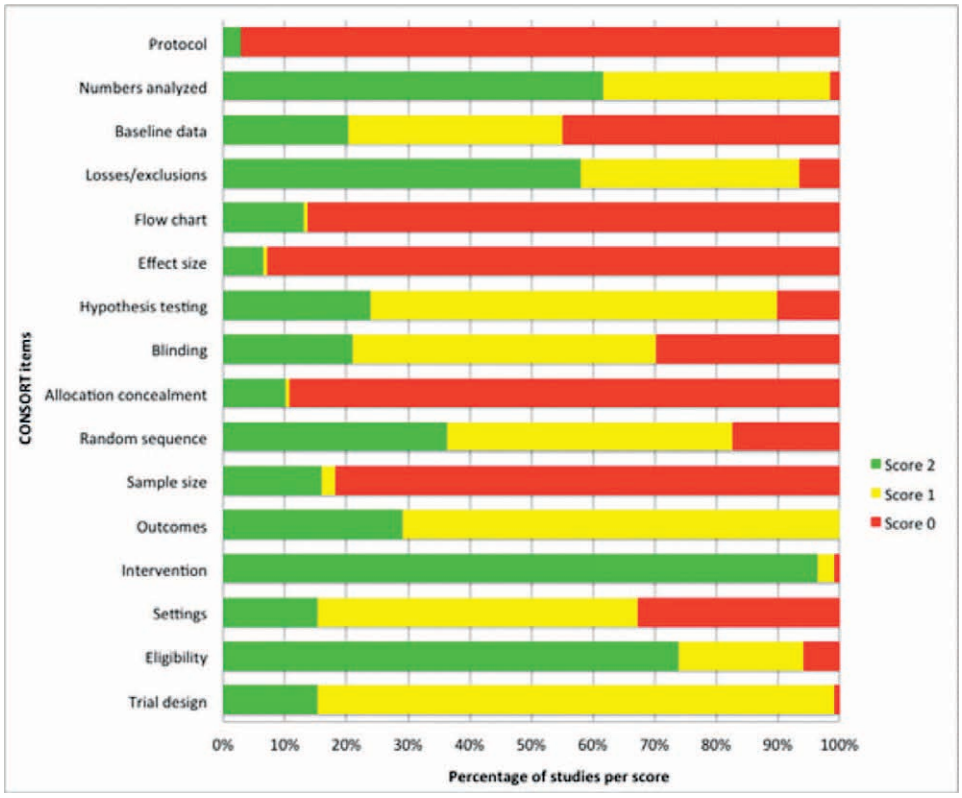

Figure 2. *Percentage of studies per CONSORT score for each CONSORT item analyzed.*

Table 3: *Characteristics of the Included Studies by Categories*

Characteristics	Categories	Number of Studies	Percentage (%)
Journal	*Dental Materials*	7	5.1
	Journal of the American Dental Association	12	8.7
	Journal of Adhesive Dentistry	14	10.1
	Journal of Dentistry	13	9.4
	Clinical Oral Investigations	14	10.1
	American Journal of Dentistry	17	12.3
	Operative Dentistry	24	17.4
	Others[a]	37	26.9
Country	Egypt	5	3.6
	Sweden	6	4.3
	Germany	8	5.8
	Belgium	11	8.0
	Turkey	15	10.9
	USA	25	18.1
	Brazil	43	31.2
	Others[b]	25	18.1
Period of time	1996-2000	11	8.0
	2001-2005	27	19.6
	2006-2010	38	27.5
	2011-2016	62	44.9
Follow-up period (years)	0.5	8	5.8
	1	24	17.4
	1.5	17	12.3
	2	37	26.8
	3	29	21.0
	4	4	2.9
	5	8	5.8
	7	3	2.2
	8	4	2.9
	12	1	0.7
	13	3	2.2

[a] *Representing 16 different journals.*
[b] *Representing 14 different countries.*

represents 46.9% of the maximum CONSORT score of 32 points. No influence of the journal on the average CONSORT score was observed ($p=0.198$; Table 4). Correlation between journal impact factor and overall CONSORT score ($r=0.089$; $p=0.93$; Figure 3) was lacking. On the other hand, significant differences among countries were observed ($p<0.001$), with the average CONSORT score of Brazil being statistically higher than Egypt and Germany. Similarly, the range of year had a significant influence on the average CONSORT score. An increase in the average CONSORT score in recent years was observed ($p<0.001$; Table 4). In all other comparisons, no significant difference was detected. The individual CONSORT score for each of the included studies can be seen in Table 5.

Risk of Bias of the Included Studies

Except for the selective outcome reporting and incomplete outcome data, most of the studies were judged as unclear or at high risk of bias in the Cochrane Collaboration tool domains (Figure 4). For the new domain included by the review authors (experimental unit), the percentage of studies at high risk of bias was even higher than the other domains (Figure 4).

Table 5 reports the individual risk of bias in each domain for all included studies. This table allows the analysis of the risk of bias within studies. Only six included studies (4.3%) were judged to be at low risk of bias in all domains. Fifty studies had unclear risk of bias in at least one domain, resulting in 36.2% of the studies being classified as having unclear risk of bias. The remaining 82 studies were at high risk of bias in at least one domain, representing 59.4% of studies at high risk of bias.

DISCUSSION

A very comprehensive search was performed, including different electronic databases and using controlled vocabulary and keywords for each of the concepts of the search. However, one cannot deny that some articles might have been missed during the search process. It is likely, however, that missed articles represent a small percentage of the included studies and, if there are any, they are unlikely to change the results presented herein.

Study Compliance With the CONSORT

The reporting quality of RCTs of adhesive systems placed in the NCCLs was assessed using an instrument tool, which was elaborated based on the CONSORT statement.[7,8] Different from earlier studies on the same topic,[11-13,15-17] the items related to the title and abstract, introduction, and discussion were not evaluated because these items are very subjective, and the study adherence to these items does not weaken either the quality of the study or their risk of bias.

The CONSORT statement reports only the items that should be addressed, but the instrument herein developed allows each item of the CONSORT statement to be scored as either 0 (no report), 1 (poor reporting), or 2 (adequate reporting), based on

Table 4: *Average CONSORT Score per Journal, Country, and Period of Time*

Characteristics	Categories	Mean ± SD	Median (interquartile range) *	*p* value[a]
Journal	*Dental Materials*	16.3 ± 5.6	16 (13.5-16) A	0.198
	Journal of the American Dental Association	15.5 ± 3.1	15.5 (13-17.5) A	
	Journal of Adhesive Dentistry	14.4 ± 3.3	15 (12-15.25) A	
	Journal of Dentistry	17.0 ± 5.0	15.5 (13-21) A	
	Clinical Oral Investigations	14.6 ± 3.5	14 (11-18) A	
	American Journal of Dentistry	13.7 ± 5.0	12 (10-17.5) A	
	Operative Dentistry	16.3 ± 1.7	16.5 (15-17.5) A	
	Others[a]	14.8 ± 5.9	14 (11-17) A	
Country	Egypt	11.2 ± 1.3	11 (10-12.25) B	<0.001
	Sweden	12.8 ± 2.7	12.5 (10-16) A,B	
	Germany	10.9 ± 3.0	10 (8.5-12.5) A	
	Belgium	15.3 ± 3.3	16 (14.25-17.75) A,B	
	Turkey	15.9 ± 3.3	15 (14-17.5) A,B	
	USA	13.4 ± 4.2	12 (12-14) A,B	
	Brazil	17.6 ± 5.2	17 (14-21) A	
	Others[b]	14.2 ± 5.0	13 (11-17) A,B	
Period of time	1996-2000	8.9 ± 2.5	10 (8-10.75) C	<0.001
	2001-2005	12.3 ± 2.2	12 (11-14) B,C	
	2006-2010	14.2 ± 2.8	14 (12-16) B	
	2011-2016	17.9 ± 5.0	17 (14-22) A	

[a] *Representing 16 different journals.*
[b] *Representing 14 different countries.*
(*) Values identified with same letters are statistically similar. Comparison are only valid for each characteristic (Kruskall-Wallis and Mann-Whitney tests).

the detailed descriptions of what should be observed in each item. This allowed a better reproducibility of the scoring process and may aid researchers to better understand what and how data should be described in future RCTs of the bonding area.

The present study observed that most of the included articles did not strictly follow the CONSORT statement. On average, a study compliance of only 46.9% with the evaluated CONSORT items was observed. An increased compliance with the CON-

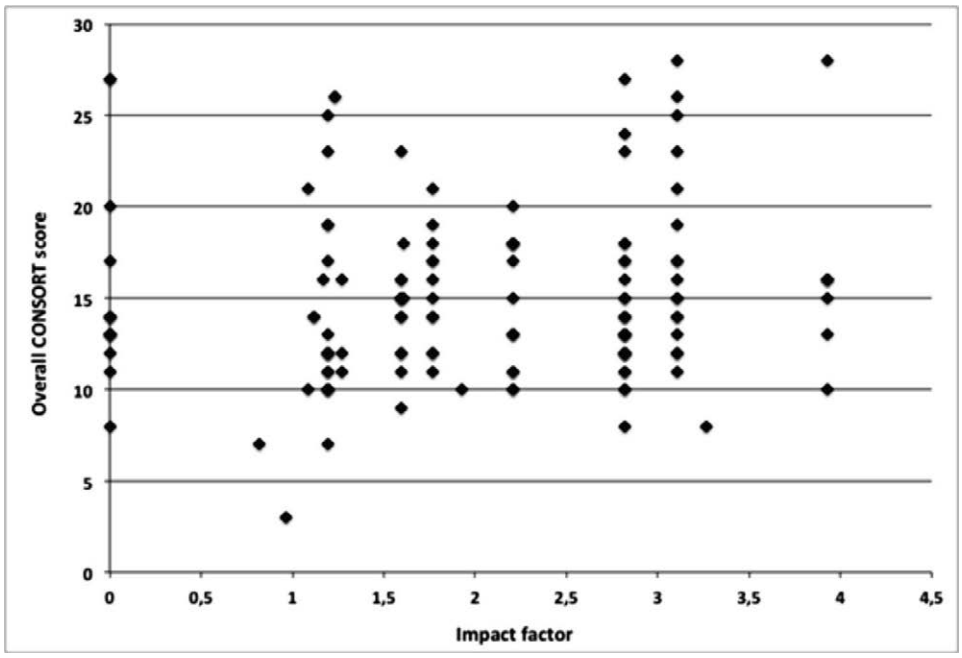

Figure 3. *Dispersion chart showing the weak correlation between journal impact factor and the overall CONSORT score.*

Table 5: *List of the Scored Papers Along With Their Average CONSORT Score and Evaluation of the Risk of bias in Each Domain*

Study identification	Year	Journal	Average CONSORT score	RISK OF BIAS TOOL					
				Random sequence	Allocation concealment	Examiner's blinding	Incomplete outcome data	Selective reporting	Experimental unit
Abdalla[32]	2008	Int J Clin Dent	12	UN	UN	L	L	L	H
Abdalla, Garcia Godoy[33]	2007	J Dent	13	UN	UN	L	L	L	H
Abdalla, Garcia-Godoy[34]	2006	Am J Dent	11	UN	UN	UN	L	L	H
Adeleke, Oginni [35]	2012	J West Afr Coll Surg	13	L	UN	UN	L	L	H
Albuquerque et al.[36]	2016	Clin Oral Invest	18	L	UN	UN	L	L	H
Alhadainy, Abdalla[37]	1996	Am J Dent	10	UN	UN	UN	L	L	H
Araujo et al.[38]	2013	Braz Dent J	20	L	UN	L	L	L	H
Araújo et al.[39]	2015	J Dent	25	L	L	L	L	L	H
Aw et al.[40]	2005	JADA	18	UN	UN	L	H	L	H
Baratieri et al.[41]	2003	Oper Dent	11	UN	UN	UN	H	L	H
Bittencourt et al.[42]	2005	Acta Odontol Scand	16	UN	UN	L	L	L	L
Blunck et al. [43]	2007	J Adhes Dent	9	UN	UN	UN	L	L	L
Boghosian[44]	1996	Compend Contin Educ	8	UN	UN	UN	UN	L	L
Bracket et al.[45]	2010	Oper Dent	12	UN	UN	L	L	L	H
Bracket et al.[46]	2002	Oper Dent	12	UN	UN	L	L	L	H
Bracket et al.[47]	2005	Oper Dent	12	UN	UN	L	L	L	H
Bracket et al.[48]	2003	Oper Dent	12	UN	UN	L	L	L	H
Bracket et al. b[49]	2002	Oper Dent	12	UN	UN	L	L	L	L
Burgess et al.[50]	2004	Am J Dent	7	UN	UN	L	L	L	L
Burgess et al.[51]	2013	Oper Dent	15	UN	UN	L	L	L	L
Burrow, Tyas[52]	2008	Aust Dent J	11	UN	UN	UN	L	L	H
Burrow, Tyas[53]	2012	Clin Oral Invest	11	UN	UN	UN	L	L	H
Burrow, Tyas [54]	2007	Oper Dent	16	UN	UN	UN	L	L	H
Burrow, Tyas[55]	1999	Am J Dent	10	H	H	UN	L	L	H
Can Say et al. [56]	2014	Clin Oral Invest	18	L	UN	L	L	L	H
Can Say et al. b[57]	2014	Dent Mater J	21	L	UN	L	L	L	H
Carvalho et al.[58]	2015	J Adhes Dent	16	L	UN	L	UN	L	H
Celik et al.[59]	2015	J Adhes Dent	23	L	UN	L	L	L	H
Celik et al. [60]	2007	Oper Dent	14	UN	UN	UN	H	L	H
Costa et al.[61]	2014	Am J Dent	23	L	L	L	L	L	L
Costa et al.[62]	2013	J Esthet Rest Dent	26	L	L	L	L	L	L
Dalkilic, Omurlu[63]	2012	J Appl Oral Sci	14	UN	UN	UN	H	L	H
Dall'Orologio[64]	2014	Am J Dent	25	L	L	UN	L	L	L
Daudt et al. [65]	2013	J Adhes Dent	15	UN	UN	L	L	L	H
Dutra-Correa et al.[66]	2013	J Adhes Dent	11	UN	UN	L	L	L	H
Ermis et al. [67]	2012	Clin Oral Invest	20	L	UN	L	L	L	L
Fagundes et al.[68]	2014	Oper Dent	11	UN	UN	L	L	L	H
Farias et al. [69]	2015	Clin Oral Invest	13	UN	UN	L	L	L	H
Farias et al. [70]	2011	Int J Braz Dent	13	UN	UN	L	L	L	H
Faye et al. [71]	2015	Int J Dent	13	UN	UN	L	H	L	H
Federlin et al.[72]	2008	Clin Oral Invest	10	UN	UN	UN	UN	L	L
Folwaczny et al.[73]	2001	Clin Oral Invest	10	UN	UN	L	L	L	H
Folwaczny et al.[74]	2001	Am J Dent	12	UN	UN	L	L	L	H
Folwaczny et al. [75]	2000	Oper Dent	8	UN	UN	L	UN	L	H
Franco et al.[76]	2006	Oper Dent	10	UN	UN	H	L	L	H
Fron et al.[77]	2011	Dent Mater	28	L	UN	L	L	L	L
Gallo et al.[78]	2005	Oper Dent	10	UN	UN	UN	L	L	L
Ghavamnasiri et al. [79]	2012	Eur J Prosth and Rest	11	H	H	UN	L	L	H
Hafer et al.[80]	2015	J Dent	17	UN	UN	L	L	L	L
Horsted-Bindslev et al.[81]	1996	Am J Dent	12	L	UN	UN	L	L	H
Karaman et al.[82]	2012	J Adhes Dent	15	UN	UN	UN	L	L	H
Kim et al.[83]	2009	Oper Dent	17	UN	UN	L	L	L	L
Kubo et al.[82]	2010	J Dent	15	L	UN	L	L	L	H
Kubo et al. [84]	2009	J Dent	14	UN	UN	L	L	L	H

Table 5: *List of the Scored Papers Along With Their Average CONSORT Score and Evaluation of the Risk of bias in Each Domain (cont.)*

Study identification	Year	Journal	Average CONSORT score	RISK OF BIAS TOOL					
				Random sequence	Allocation concealment	Examiner's blinding	Incomplete outcome data	Selective reporting	Experimental unit
Kubo et al. [85]	2006	J Dent	14	UN	UN	L	L	L	H
Kurokawa et al.[86]	2007	Dent Mater J	10	UN	UN	UN	L	L	H
Lawson et al. [87]	2015	J Dent	23	L	L	L	L	L	L
Loguercio et al. [88]	2015	J Dent	26	L	L	L	L	L	H
Loguercio et al.[89]	2007	JADA	17	L	UN	L	H	L	L
Loguercio et al.[90]	2010	Oper Dent	18	L	UN	L	L	L	L
Loguercio et al.[91]	2011	Clin Oral Invest	18	L	UN	L	L	L	L
Loguercio et al.[92]	2015	Oper Dent	23	L	L	L	L	L	L
Loguercio et al. [93]	2006	J Adhes Dent	15	UN	UN	L	L	L	L
Loguercio et al. [94]	2005	Clin Oral Invest	11	UN	UN	L	UN	L	L
Loguercio, Reis[95]	2008	JADA	19	L	UN	L	L	L	L
Luque-Martinez et al. [96]	2015	J Dent	28	L	L	L	L	L	L
Matis et al. [97]	2004	J Am Dent Assoc	14	UN	UN	UN	L	L	L
McCoy et al. [98]	1998	JADA	11	UN	UN	UN	UN	L	H
Mena Serrano et al.[99]	2013	J Esthet Rest Dent	26	L	L	L	L	L	H
Merte et al. [100]	2000	J Biomed Mater Res	8	UN	UN	UN	L	L	H
Montagner et al.[101]	2015	Braz Dent J	27	L	L	L	L	L	H
Moosavi et al.[102]	2013	Oper Dent	18	UN	UN	L	L	L	L
Moretto et al. [103]	2013	J Dent	21	L	UN	L	L	L	L
Mortazavi et al. [104]	2012	Dent Res J	17	UN	UN	L	L	L	L
Neoet al. [105]	1996	Am J Dent	11	UN	UN	UN	L	L	H
Oliveira et al.[106]	2012	Int J Clin Dent	14	UN	UN	L	L	L	H
Onal, Pamir [107]	2005	J Am Dent Assoc	14	H	H	UN	L	L	H
Ozel et al.[108]	2010	Aust Dent J	12	UN	UN	L	L	L	H
Paula et al.[109]	2015	Int J Esthet Dent	27	L	L	L	L	L	H
Pena et al.[110]	2016	Oper Dent	14	L	UN	L	L	L	H
Perdigão et al.[111]	2005	Am J Dent	12	UN	UN	L	L	L	H
Perdigão et al.[112]	2005	J Adhes Dent	12	UN	UN	L	L	L	H
Perdigão et al.[113]	2012	Oper Dent	13	UN	UN	L	L	L	H
Perdigão et al.[114]	2001	J Adhes Dent	14	UN	UN	L	L	L	H
Perdigão et al. [115]	2014	Oper Dent	27	L	L	L	L	L	H
Perdigão et al. [116]	2004	Compend Cont Educ Dent	14	UN	UN	L	L	L	H
Peumans et al.[117]	2007	J Adhes Dent	14	UN	UN	L	L	L	H
Peumans et al.[118]	2015	Dent Mater	16	UN	UN	L	L	L	H
Peumans et al.[119]	2007	Dent Mater	16	UN	UN	L	L	L	L
Peumans et al.[120]	2010	Dent Mater	15	L	UN	L	L	L	H
Peumans et al. [121]	2005	Eur J Oral Sci	15	UN	UN	L	L	L	H
Peumans et al. [122]	2012	Clin Oral Invest	17	L	UN	L	L	L	L
Pollington, Van Noort[123]	2008	Am J Dent	13	L	UN	UN	UN	UN	L
Qin et al.[124]	2013	Clin Oral Invest	13	UN	UN	L	L	L	H
Reis et al.[125]	2006	Oper Dent	14	UN	UN	L	L	L	H
Reis et al.[126]	2010	Am J Dent	19	L	UN	L	L	L	L
Reis et al. [127]	2009	JADA	21	L	UN	L	L	L	L
Reis, Loguercio [128]	2009	Oper Dent	17	UN	UN	L	L	L	L
Ritter et al.[129]	2008	Oper Dent	13	UN	UN	UN	L	L	H
Ritter et al. [130]	2009	JADA	16	H	UN	UN	L	L	H
Saboia et al. [131]	2006	Oper Dent	13	UN	UN	L	L	L	H
Sakrana et al.[132]	2004	J Oral Rehabil	10	UN	UN	L	H	L	H
Santiago et al.[133]	2010	Braz Dent J	13	UN	UN	L	L	L	H
Santiago et al.[134]	2003	J Appl Oral Sci	14	UN	UN	UN	L	L	H
Sartori et al.[135]	2012	J Adhes Dent	12	UN	UN	L	L	L	H
Sartori et al. [136]	2013	Oper Dent	15	L	UN	L	L	L	H
Sartori et al. [137]	2013	J Dent	17	UN	UN	L	L	L	H
Schattenberg et al. [138]	2008	Clin Oral Invest	13	UN	UN	UN	L	L	H

Table 5: *List of the Scored Papers Along With Their Average CONSORT Score and Evaluation of the Risk of bias in Each Domain (cont.)*

Study identification	Year	Journal	Average CONSORT score	RISK OF BIAS TOOL					
				Random sequence	Allocation concealment	Examiner's blinding	Incomplete outcome data	Selective reporting	Experimental unit
Scotti et al. [139]	2016	Am J Dent	19	L	UN	L	L	L	H
Soderholm et al.[140]	2013	Am J Dent	12	UN	UN	UN	L	L	H
Souza et al.[141]	2014	J Conserv Dent	14	L	UN	L	L	L	H
Stojanac et al.[142]	2013	Oper Dent	13	UN	UN	UN	L	L	L
Swift et al. [143]	2001	J Dent	12	UN	UN	UN	L	L	H
Swift et al. [144]	2001	JADA	12	UN	UN	UN	L	L	H
Torres et al. [145]	2014	J Dent	15	UN	UN	L	L	L	H
Tuncer et al.[146]	2013	Aust Dent J	16	L	UN	L	L	L	H
Turkun[147]	2003	J Dent	11	UN	UN	UN	L	L	H
Turkun[148]	2005	JADA	15	UN	UN	H	L	L	H
Turkun, Celik[149]	2008	J Adhes Dent	16	UN	UN	UN	L	L	H
Tyas[150]	1996	Int Dent J	3	UN	UN	UN	UN	L	H
Tyas, Burrow [151]	2002	Am J Dent	10	UN	UN	UN	L	H	H
Van Dijken[152]	2013	Dent Mater	16	UN	UN	UN	L	L	H
Van Dijken[153]	2007	J Adhes Dent	12	UN	UN	UN	L	L	H
Van Dijken [154]	2004	Am J Dent	10	UN	UN	UN	L	L	H
Van Dijken [155]	2010	Dent Mater	13	UN	UN	UN	L	L	H
van Dijken[156]	2000	Dent Mater	10	UN	UN	UN	L	L	H
Van Dijken, Pallesen[157]	2012	J Dent	16	L	L	L	L	L	H
Van Landuyt et al.[158]	2011	Eur J Oral Sci	18	L	UN	L	L	L	L
Van Landuyt et al. [159]	2008	J Dent	19	L	UN	L	L	L	L
Van Landyut et al.[160]	2014	Clin Oral Invest	18	L	UN	L	L	L	L
Van Meerbeek et al.[161]	2004	Oper Dent	13	UN	UN	L	UN	L	L
Van Meerbeek et al.[162]	1996	Quintessence Int	7	UN	UN	L	UN	L	H
Wilder et al.[163]	2009	JADA	12	UN	UN	UN	H	L	H
Yaman et al. [164]	2014	Clin Oral Invest	15	L	UN	L	L	L	H
Yazici et al. [165]	2010	J Adhes Dent	15	L	UN	L	H	L	H
Zander Grande et al.[166]	2011	JADA	17	UN	UN	L	L	L	L
Zander-Grande et al.[167]	2014	Oper Dent	24	L	L	L	L	L	L
Zhou et al.[168]	2009	Am J Dent	17	L	UN	L	L	L	L

UN – unclear risk of bias; L – low risk of bias; H – high risk of bias

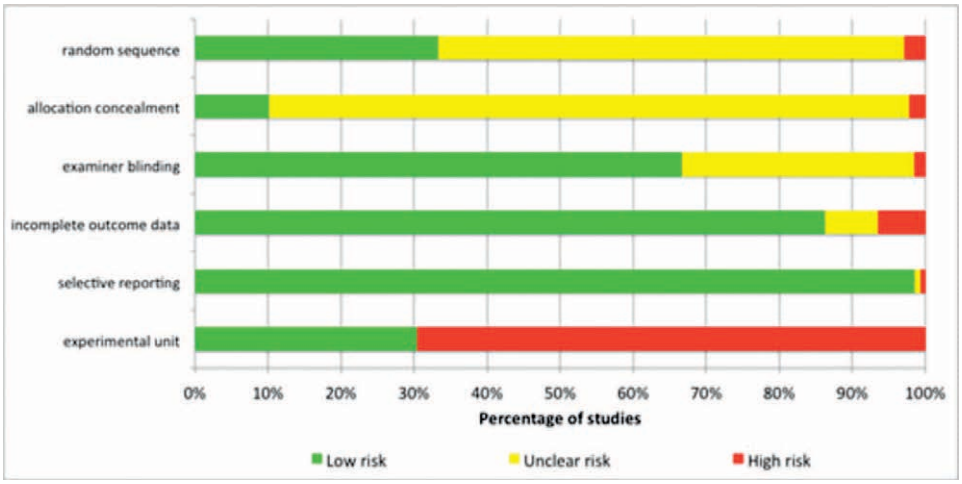

Figure 4. *Methodologic risk of bias chart.*

SORT statement was observed in the last 6 years (mean CONSORT score of 17.9 ± 5.0; 49% compliance), a finding already observed by other authors.[14,15] However, this increase is still trivial and substandard because it reached approximately a little more than half of the maximum CONSORT score of 32 points.

Compliance with the CONSORT statement has already been studied in other fields of dentistry. In the orthodontic area, studies reported a compliance of 41.5%,[15] 51.7%,[14] and 68.9%.[16] In the fields of prosthodontics and implant dentistry, a compliance of approximately 68% was observed.[11] Variations within the same area are likely related to the inclusion criteria of the studies, mainly regarding their period of publication. Additionally, variations in the approach used to evaluate the CONSORT compliance can yield discrepancies in the results. However, regardless of these variations, one may see that even in the best situation the compliance was still low, indicating need of improvement.

It has already been reported that journal endorsement of the CONSORT statement might beneficially influence the completeness of RCTs reporting in medical journals[10] and in orthodontic dentistry journals.[15,19] Although some of the main journals that published studies of adhesives in NCCLs endorsed the CONSORT Statement (ie, *Journal of the American Dental Association, Journal of Dentistry,* and *American Journal of Dentistry*), a journal and its impact factor did not influence the average CONSORT score, neither in the present study nor in a systematic review in medicine.[9] Sarkis-Onofre and others[20] recently confirmed no correlation exists between journal endorsement of the CONSORT statement with improved completeness of RCTs reporting in restorative dentistry. Perhaps editors and editorial boards from these journals do not check the submitted articles against the CONSORT statement, which prevents the journals from reaching the expected benefits. More attention to these items during the peer-review process is required.

As reported in the results section, the item's sample size, allocation concealment, effect size, flow chart, and protocol were the aspects with poorest reporting. A priori sample size calculation prevents the publication of underpowered RCTs. In underpowered studies, negative findings do not necessarily mean the groups are not different from one another; it may be the result of sample size being too small to detect a "clinically important difference" among the groups.

A study should involve a sample size large enough to have a high probability (power) of detecting as statistically significant a clinically important difference of a given size, if such a difference exists. For such a purpose, and in superiority trials, authors should describe 1) the estimated outcomes in each group for the primary outcome(s) (ie, the clinically important difference between groups); 2) type I error; 3) power; and 4) for continuous outcomes, the standard deviation of the measurements.

In the present study, approximately 82% of the RCTs did not report sample size calculation at all. This is also problematic in the medical field. For instance, Chan and Altman[21] reported that 73% of the 519 medical trials indexed in PubMed in December 2000 did not report sample size calculation. To make the scenario even worse, authors usually do not report the primary outcome for which the sample size calculation was performed. In this review, only 30% of the included RCTs reported the primary outcomes of the study clearly. Although, the United States Public Health Service evaluation[22] and more recently the Fédération Dentaire Internationale criteria[23] contain several criteria to be evaluated, in the case of RCTs about adhesive systems in NCCLs, retention rate should be regarded as a primary outcome and used for sample size calculation for being a true end point.

The reporting of the randomization process should include details about the methods used to generate the random sequence. In this review, it was observed that this item was reported inadequately, or it was not reported at all in 63.8% of the cases. In the fields of prosthodontics and implant dentistry, this figure was 44.3%.[11] Usually, authors refer to terms such as "random allocation" or "the groups were randomized," without further elaboration. Authors should specify the method of sequence generation (such as a random number table or a computerized random number generator, coin toss, and dice throwing), as well as restrictions to the process such as stratification and block randomization.

Allocation concealment seeks to prevent foreknowledge of the sequence generation before implementation, and it is as important as sequence generation to prevent selection bias. Allocation concealment can always be successfully implemented. It should not be confused with blinding, as blinding prevents performance and detection bias.[24] Despite the importance of allocation concealment, one can observe in 89.1% of the cases that there was no description of this item at all. This is also in agreement with previous literature findings. An

inadequacy of allocation concealment description was observed in 78% of the RCTs among dental journals[25] and 93% in the specialty of periodontology.[13] Another problem related to inadequately and unclearly concealed RCTs is that effect sizes are exaggerated in favor of the experimental group.[4]

Blinding is also a key element in RCT reporting. In the present review, 70% of the RCTs performed poor or no reporting of blinding. During the execution of RCTs in NCCLs about adhesive systems or composite resins, operator blinding is quite impossible. However, patient and evaluator can still be blinded. If the primary outcome is retention rate, which is an objective parameter, lack of evaluator blindness does not put the study at high risk of bias, but for other subjective criteria such as marginal discoloration, marginal adaptation, color match, and others, the lack of evaluator blinding puts the study at high risk of bias. Patient blinding is especially important when patient-centered subjective outcomes such as pain scores are collected, as they are more prone to bias. This is the case when different desensitizers are evaluated in NCCLs. In summary, blinding of the patients and the treatment providers may not always be possible; however, blinding of the evaluators and the analysts may.

One of the common failures during reporting of blinding is that authors usually report "this study was single-blind" or "this was a double-blind study," without reporting who was blinded; this should be clearly stated in the RCTs. In agreement with these findings, Pandis and others[25] reported that inadequate description of blinding in RCTs published in leading dental journals ranged from 74% to 100%. In implant dentistry, the lack of adequate blinding reporting was informed to be 58%.[26]

Reporting of effect size and confidence intervals facilitates interpretation of important clinical differences. Hypothesis testing with p values and statistical significance is based on arbitrary cutoff points (ie, 0.05) and are sensitive to sample size and variance. By increasing sample size, very small and unimportant clinical differences may become statistically significant and may be erroneously interpreted as being "clinically" important.[24]

In this study, 92.8% of the RCTs did not describe any effect size for at least the primary outcome. This is also a problem in medical journals.[27] Authors should report an estimate of the treatment effect, which is a contrast between the outcomes in the comparison groups. For binary outcomes, the effect size could be the risk ratio (relative risk), odds ratio, or risk difference; for survival time data, it could be the hazard ratio or difference in median survival time; and for continuous data, it is usually the difference in means or standardized difference in means. Confidence intervals should be presented as they provide information about data precision.

The lack of description of effect sizes suggests that authors still rely on hypothesis testing for group comparisons. Researchers are advised to move away from significance tests to effect size reporting, delimited by confidence intervals. This method incorporates all the information normally included in a hypothesis but in a way that emphasizes the size of the difference (clinical significance rather than statistical significance).[27,28]

The design and conduct of some RCTs may be not straightforward, particularly when there are losses to follow-up or exclusions. This prevents the description of the numbers of participants through each phase of the study in a few sentences. In complex studies, it may be challenging for readers to discern whether and why some participants did not receive the treatment as allocated or if they were lost to follow-up or were excluded from the analysis.[29] This can be simply described by introducing a flow chart with the number of participants in each phase of the trial. Although the CONSORT Statement recommends the inclusion of a flow chart, only 13% of the RCTs herein evaluated followed this recommendation.

Another type of bias commonly faced in RCTs is selective outcome reporting. As pointed out in an editorial by de Angelis and others,[30] researchers (and journal editors) are generally most enthusiastic about the publication of RCTs that show either a large effect of a new treatment (positive trials) or equivalence of two approaches to treatment (noninferiority trials). Less excitement is observed in RCTs that show that a new treatment is inferior to standard treatment (negative trials), and researchers show even less interest in RCTs that are neither clearly positive nor clearly negative because inconclusive RCTs will not, by themselves, encourage changing practices. Additionally, sponsored RCTs are likely to remain unpublished if the results of the RCTs place financial interests at risk.[30]

To manage such problems, the International Committee of Medical Journal Editors (ICMJE) proposes comprehensive trials registration. Trials must register at or before the onset of patient enrollment.[30] For the ICMJE, this policy applies to any clinical trial that started enrollment after July

1, 2005. However, only 4 of 110 included studies of this review published in 2005 or later performed trial registration (Table 5). Authors are advised to perform trial registry due to its advantages: 1) selective reporting can be avoided and if present, could be checked by comparing the published version of the paper with their registered protocol; and 2) it reduces publication bias, as studies with negative or inconclusive findings would be available for evaluation. Some dentistry journals such as *Journal of Dentistry* and *Operative Dentistry* have added this indication as mandatory in their instructions for authors.

Other items of CONSORT such as numbers analyzed, baseline data, losses and exclusions, outcomes, setting, and trial design deserves some discussion. Regarding numbers analyzed, the number of participants per group in all analyses should be clear in the study. Reporting summary statistics or only percentages, relative risks, or odds ratios is not enough as they do not allow readers to assess whether some of the randomly assigned participants were excluded from the analysis. The same should be applied to losses and exclusions. Along with the description of these figures per group, reasons for the losses and exclusions should be given as they may be related to the intervention. For instance, when a patient moves to another city, it is unlikely to be related to the intervention, but if a patient does not attend the recalls because he or she wants to be withdrawn from the trial, then the reason may be related to side effects or lack of efficacy of the treatments under evaluation.

Baseline information, adequately reported in only 20.3% of the papers, allows readers to check whether groups are comparable at baseline. Although proper random assignment prevents selection bias, it does not guarantee that the groups are equivalent at baseline. Any differences in baseline characteristics are, however, the result of chance rather than bias: the reason why there is no need to perform hypothesis testing for these characteristics. For instance, in the case of RCTs in NCCLs, the presence of occlusal wear facets is considered a predictive factor for restoration loss. The number of restorations placed in teeth with or without occlusal wear facets per group is therefore essential for baseline evaluation.[31]

For all three of these items (numbers analyzed, losses and exclusions, and baseline characteristics), authors should be careful when presenting data. First, displaying percentages instead of raw numbers is risky. Rounded percentages may be compatible with more than one numerator and if authors fail to provide the number analyzed, then the denominator (total number of participants evaluated) will be unclear. For instance, 50% may represent five of 10 but also 500 of 1000. Second, merged data of groups can be provided as long as their individual values are also reported. Third, continuous variables should be presented as means and standard deviations (or standard errors) or medians and interquartile ranges (when not normally distributed); dichotomous variables in number of counts versus total number of observations.

The trial design involves the description of type of the trial (parallel, cross-over, factorial, split-mouth, and/or multiple restorations); the conceptual framework (superiority, noninferiority, or equivalence trial); and the allocation ratio (eg, 1:1 or 1:2). The setting (where and when the study was performed) is also essential to place a study in historical context and to evaluate its external validity (generalization of the findings to other populations).

Risk of Bias

Except for incomplete outcome data and selective reporting, which is not a major problem in the included articles of the present studies, in all other domains of the CCRT, RCTs were judged to have unclear or high risk of bias. The implications of inadequate sequence generation, allocation concealment, and examiner blinding were already discussed in detail.

We also added another domain in the CCRT for the analysis of the risk of bias, which is the experimental unit. The great majority of the authors placed multiple restorations per patient and considered each tooth as an experimental unit, without taking into consideration the clustered nature of the data. In these cases, authors applied conventional hypothesis testing statistics that assume that data are "independent." Treating multiple observations from one participant as independent data is a serious error. Having this in mind, authors are advised to 1) place a single restoration per group in each patient in a paired design; 2) place more than one restoration per group in each patient, but only one value (median, mean, worst score, etc) per patient/group should be statistically analyzed; or 3) place multiple restorations per patient but use more advanced statistical models to account for the paired nature of the data.

In general, only 4.3% of the studies were considered at low risk of bias in this item. Most of the studies (59.4%) were at high risk of bias, and this

affects the quality of the body of evidence produced thus far.

Although some journals have adopted the CONSORT guidelines in the instructions for authors, active compliance is yet to be achieved. Perhaps the inclusion of additional subheadings for RCTs, as suggested by Kloukos and others,[11] could result in better compliance with the CONSORT statement. The results of the present study indicate that adherence of RCTs that evaluate adhesive systems in NCCLs to the CONSORT statement requires improvements. Adherence to the CONSORT statement will also reduce the high risk of bias of studies in the field.

Acknowledgements

This study was partially supported by CAPES and National Council for Scientific and Technological Development (CNPq) under Grants 304104/2013-9 and 305588/2014-1.

Conflict of Interest Declaration

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

(Accepted 9 August 2017)

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©Operative Dentistry, 2018, **43-3,** E152-E157

Diagnosis of Pit-and-fissure Caries Using Three-dimensional Scanned Images

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Clinical Relevance

Detecting occlusal caries with scanned three-dimensional images is as accurate as visual examination with magnification and appropriate lighting.

SUMMARY

Diagnosis of the extent of pit-and-fissure caries has been subjective and thus difficult to teach and categorize for treatment planning. This *in vitro* study compares occlusal caries diagnosis of extracted posterior teeth (n=49) using three-dimensional (3D) scanned images vs visual examination, according to the International Caries Detection and Assessment System (ICDAS). The surfaces chosen for study represent all ICDAS classifications. Five experienced restorative faculty members examined scanned images for 60 seconds from a standardized series of views of each surface and scored them independently. One month later, the same teeth were examined visually by the same five raters with magnification and LED headlamps, with compressed air available. Intrarater and interrater agreement and validity were assessed using intraclass correlation coefficients (ICCs). The ICCs, ranging from 0.90 to 0.93, indicated excellent agreement between and within raters and between the raters and the gold standard ICDAS determination. This suggests that both photographs and 3D scans of pits and fissures are equally effective in diagnosing caries.

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DOI: 10.2341/17-076-L

INTRODUCTION

Clinicians have long noted the difficulty of accurately assessing the extent of pit-and-fissure caries.[1] Carious lesions exist on a continuum of severity from early decalcification to complete destruction of the clinical crown and root. A standard descriptive framework that reasonably predicts the amount of carious tissue destruction is accordingly required to develop an appropriate treatment plan. While the amount of physical cavitation of the lesion has been proposed[2] as the gold standard in evaluating smooth surface caries, and although the extent of damage can be evaluated on standard radiographs, predicting caries level in occlusal pit-and-fissure caries has

proven more problematic. It has been suggested that extensive fluoridation has created enamel that is more resistant to acid attack, delaying frank cavitation even when dentin caries has progressed.[3] The traditional technique of caries detection taught to generations of clinicians involves forcing a sharp explorer into the suspect fissure and testing for resistance to withdrawing ("a stick"). This technique has been long discredited, but it still persists in practice.[4,5] The standard operative classification proposed by GV Black in the 1800s is not based upon describing the lesion extent or activity. Instead, it references the planned location of the restoration.

An alternate system using visual diagnostic criteria for caries is defined in the International Caries Detection and Assessment System (ICDAS II), which was developed as a tool for epidemiology and research and has also been accepted as a formal curricular framework for teaching in the United States.[6] This system has been validated through comparison to histologic, radiologic, and fluorescence-based findings and was shown to correlate more highly than radiographs in treatment decisions made by experienced practitioners.[7-11,12] The basis of the ICDAS system is careful visual evaluation of the fissure system with magnification to 2.5×, as stronger magnification has been shown to decrease the specificity of examination to unacceptable levels.[13] Intraoral photographs of teeth have been compared with visual appearance of teeth on five- to 11-year-olds and have been found to be equivalent for teaching, epidemiologic, and research purposes.[14] Visual exam with ICDAS has also been shown to be superior to radiographs in detecting occlusal caries.[11]

The widespread use of three-dimensional (3D) digital imaging devices in clinical care for documentation, patient education, and creation of impressions has created an opportunity to use these images for diagnosis as well. One pilot study[12] investigating the feasibility of teledentistry in remote pediatric dental evaluation found no difference in dmft/DMFT scores between clinical exams and photos made with a two-dimensional intraoral camera. Electronic 3D images have potential for use in creating realistic teaching cases for students, in which the images could be manipulated in visual space. Since images are routinely transmitted electronically for laboratory fabrication of restoration it should be possible to use them to document caries for insurance purposes, especially in the early stages, during which they are more accurate than radiographs.[12] To date, no study has looked at 3D imaging when compared to clinical appearance in ICDAS scoring. That was the purpose of this study.

METHODS AND MATERIALS

Sample Selection and Classification

A sample size calculation indicated that, with 95% confidence, having five raters examine 49 teeth would yield estimated intrarater (IAR) and interrater (IER) agreement values that were within 0.10 of the true values, assuming that the true repeatability was 0.71 or greater. Thus, in this institutional review board–exempt study, seven permanent teeth from each ICDAS category (see Table 1; 49 teeth total) were selected from a pool of recently extracted posterior teeth without restorations or fluorosis.

The teeth were cleaned of calculus and debris using a scaler and toothbrush under running water and were then stored in 0.9% sodium chloride/0.2% sodium azide solution at 36°F. Using 2.5× magnifying dental loupes (Designs for Vision Inc, Long Island, NY, USA) and headlamps (Ultra-Light Optics, Fountain Valley, CA, USA), the teeth were then categorized by two senior educators experienced in teaching ICDAS. The consensus opinion of the ICDAS classifications was considered the "gold standard" reference classification for the study.

3D Scanning of Teeth

Using the 3D color imaging mode of a high-definition hand-held intraoral scanner (3Shape, Copenhagen, Denmark) images were captured per the ICDAS protocol of first examining the surface wet, then drying it with a five-second air stream, and then noting the changes between wet and dry. Six images were captured of each tooth (three wet and three dry), one of the occlusal (Figure 1), one from a slight lingual tilt (Figure 2), and one from a slight facial tilt to simulate a clinical situation. Images were processed with 3Shape Trios Version 1.3.4.5 and stored as screen shots on a MacBook Pro Retina (Apple Inc, Cupertino, CA, USA).

Data Collection

Five clinician educators experienced with the ICDAS method successfully completed online ICDAS training (www.icdas.org) for calibration. They were brought together in a room with normal lighting and shown images on a 27-inch iMac 5K (Apple) to simulate a clinical evaluation. Each tooth was represented by 3D images captured wet and dry. To simulate the clinical evaluation, the wet image was viewed first for 20 seconds by all evaluators,

Table 1: *Explanation of International Caries Detection and Assessment System (ICDAS) and Caries Classification System (CCS) Caries Classification Systems*

CCS System	ICDAS System		Characteristics	Histologic Correlation
	Merged Codes	Full Codes		
0 Sound	0 Sound	0	Same appearance wet or dry, no surface breakdown or underlying color change	No carious demineralization of enamel or dentin
1 Initial	A Initial	1	Chalkiness visible on drying, confined to fissure	Demineralization in outer half of enamel
		2	Chalkiness visible wet wider than natural fissure or groove	Demineralization in inner half of enamel, outer third of dentin
2 Moderate	B Moderate	3	Areas of localized enamel breakdown (microcavitation) present in walls or base of groove, but no discontinuity	Localized enamel breakdown, extension to middle third of dentin
		4	Shadow visible from occlusal, more easily seen when tooth wet	Middle one-third dentin ± microcavitation
3 Advanced	C Extensive	5	Carious dentin visible, but less than half of surface	Inner one-third dentin; may involve pulp
		6	Visible dentin, cavitation involving > one-half tooth surface	Inner one-third dentin; may involve pulp

Figure 1. *Occlusal view, dry. Scanned three-dimensional (3D) image of the occlusal of an ICDAS 5 lesion. Note the cavitation as well as areas of surface decalcification and shadowing in other areas visible in the scan. Original scan is in color.*

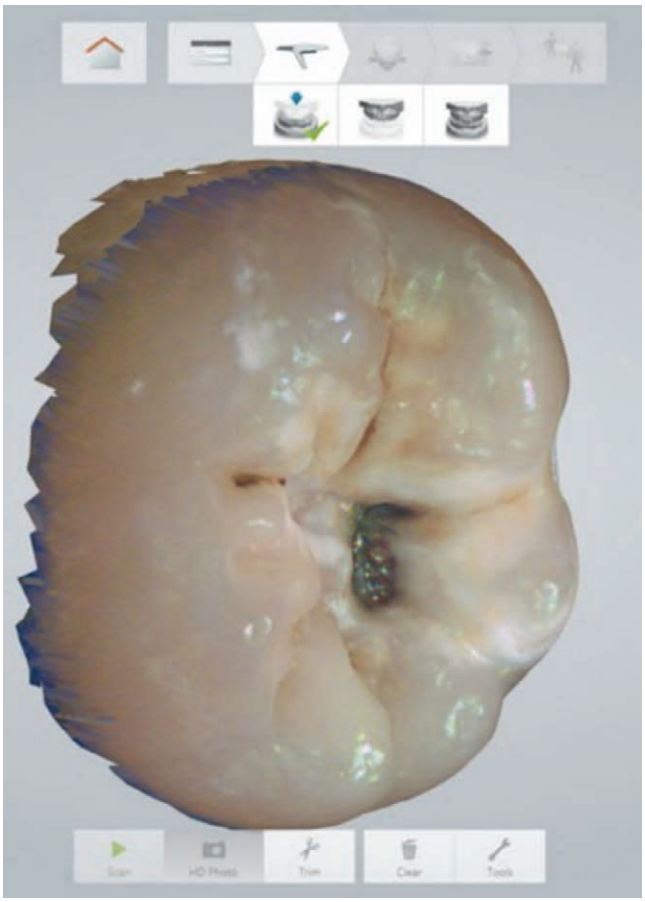

Figure 2. *Lingual tilt view, dry. Same tooth viewed from the lingual to show additional features of cavitated area and surface anatomy.*

with a standardized rotation of the 3D image of viewing from the occlusal straight on for 10 seconds, slightly to the buccal for five seconds, and slightly to the lingual for five seconds. The dry surface scan was then presented for 40 seconds with the same standardized rotation for 10 seconds for each of the three angles and a final 10 seconds on the straight occlusal image. Each evaluator independently scored the surface. This was designated the "digital" approach.

The teeth were then stored in separate numbered vials in sodium azide solution at 36°F. They were randomized according to a schedule maintained by an investigator who did not participate in the examination process.

Approximately one month later, the same investigators independently evaluated all of the teeth visually for one minute per tooth. Teeth were presented in separate cups in water, so they were examined first wet, then after a five-second air stream using a head lamp and 2.5× magnification. Each was assigned an ICDAS score based on this examination. This was designated the "analog" approach.

Statistical Analysis

To assess reliability, both IAR agreement and IER agreement were examined. IAR measures the extent to which each rater tends to agree with himself between the analog and digital approaches, regardless of individual tooth or ICDAS classification. Intraclass correlation (ICC) was used to assess the IAR for all raters combined. IER measures the extent to which the five raters tend to agree with each other, regardless of method (analog vs digital), individual tooth, or ICDAS classification. The ICC was used to assess the IER among the five raters for the analog and digital methods combined and separately for the analog and digital methods, regardless of individual tooth or ICDAS classification.

To assess validity, the ICC was used to measure agreement between the analog result and the gold-standard ICDAS classification and between the digital result and the gold-standard ICDAS classification, regardless of rater or individual tooth. Reliability and validity were assessed by calculating the appropriate ICCs after combining the classification results obtained by the five raters with the gold standard results for all 49 teeth, which consisted of seven teeth in each of the ICDAS classes 0-6.

Evaluation of the IER and IAR within each ICDAS classification was not an objective of the study and could not be attempted because the number of specimens (n=7) was too low, relative to the number of raters, to yield a meaningful assessment of reliability.

With regard to interpretation, the ICC as an agreement coefficient ranges between a maximum value of 1, which indicates "perfect agreement," and a minimum value of 0, indicating "no agreement." A value of the ICC between 0.75 and 1.00 indicates "excellent" agreement, values between 0.40 and 0.74 indicate "fair to good" agreement, and values less than 0.40 indicate "poor" agreement.[15] The method of Gilder and others was used to estimate the ICC and to find approximate 95% confidence intervals (CIs) for all agreement coefficients.[16] The CI provides information on how precise the estimated agreement coefficient is: the narrower the interval, the more precise the agreement; the wider the interval, the less precise the agreement. All calculations were performed using SAS 9.4.

RESULTS

Table 2 contains the IER and IAR agreement coefficients based upon the full ICDAS classification system. For this analysis, there were five raters, 49 specimens (ie, teeth), and two "trials" (analog and digital). All of the ICC values in Table 1 indicate "excellent" IER and IAR agreement and, hence, excellent reliability. The CIs are quite narrow, indicating a high degree of precision in the estimation of the agreement coefficients.

Table 3 contains the ICC values for agreement between results obtained using the analog method and the teeth classified using the gold-standard ICDAS determination and agreement between results obtained using the "digital" method and the gold-standard ICDAS. All of the ICC values indicate excellent agreement for both the analog and digital methods, and the CIs are quite narrow, thus establishing excellent validity for both methods.

DISCUSSION

Using visual examination of pit-and-fissure caries has been shown[6,8,18] to be clinically appropriate and effective. To describe and categorize visual evidence, two classification systems are currently in use. In addition to ICDAS, the American Dental Association has published a simplified version of the ICDAS, known as the Caries Classification System (CCS), which combines similar ICDAS criteria to create four

Table 2: *Interrater (IER) and Intrarater (IAR) Agreement Using all 49 Teeth (Seven Teeth in each International Caries Detection and Assessment System [ICDAS] Class)*

Method	No. of Specimens	Type of Agreement	ICC	95% CI
Analog only	49	IER	0.93	(0.90, 0.96)
Digital only	49	IER	0.90	(0.85, 0.94)
Digital and analog	49	IER	0.91	(0.88, 0.94)
	49	IAR	0.90	(0.87, 0.93)

Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient.

Table 3: *Agreement of Analog and Digital Methods with Gold Standard International Caries Detection and Assessment System (ICDAS) Classification Using all 49 Teeth (Seven Teeth in each ICDAS Class)*

Method	No. of Specimens	ICC	95% CI
Analog	49	0.93	(0.90, 0.95)
Digital	49	0.91	(0.88, 0.94)

Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient.

CCS categories instead of the seven used in the ICDAS. Diagnosis with visual criteria is currently included in the core curriculum framework in cariology for US dental schools, with two systems, ICDAS and CCS, currently proposed.[19,20] For the purposes of this study, ICDAS was chosen because the investigators considered it more detailed than CCS.

This study indicates that caries detection using ICDAS visual criteria with 3D scans can be as accurate as direct visual assessment. Valid and reliable diagnosis of occlusal carious lesions is an important first step in appropriate treatment planning. ICDAS visual criteria can provide information on the level of disease present clinically that, when taken in the context of the caries risk assessment of diet, salivary flow, and recent history of carious lesions, provides guidance for treatment of the patient.[11,12] If 3D images are validated as accurate representations of disease state then digital treatment planning may become a reality, with digital radiographs, occlusal scans, and digital models being used to document extent of disease.

When the entire data set of 49 teeth was analyzed, there was excellent IER and IAR agreement and excellent agreement of the ICDAS gold standard with both the analog and digital methods. This study showed that experienced evaluators are as accurate in classifying pit-and-fissure caries with scanned images as they are with direct visual exam, and this study also showed more overall consistency among raters than has been associated with most previous studies. The authors detected one outlier in each of the seven ICDAS categories, defined as the median score for that tooth differing by at least one ICDAS classification from the gold standard result. Because of this discrepancy, the authors considered eliminating these teeth from the analysis, but the agreement results after removing them (results not shown) did not differ in any meaningful way from the results based on all 49 teeth.

In the educational setting, scans may convey more information to students in teaching how to identify caries using ICDAS than other modalities, since several scan views can be evaluated to teach in a more realistic manner. There may also be a role for scans in verifying clinical data for insurance purposes, since scanned images can be transmitted digitally via e-mail. Further *in vivo* clinical studies would be helpful to confirm these findings.

CONCLUSION

Within the limitations of this *in vitro* study, caries classification of pits and fissures can be evaluated as accurately with 3D scanned images as with direct visual examination *in vitro*.

Acknowledgement

The authors gratefully acknowledge the support of Custom Automated Prosthetics, Stoneham, MA, USA, through donation of equipment and software. Dr David H Pashley is acknowledged for assistance in specimen procurement.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Medical College of Georgia. The approval code for this study is 94-02-194.

Conflict of Interest

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

(Accepted 28 August 2017)

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