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Clinical Technique/Case Report

Elevation of an Amalgam-stained Margin With Resin-modified Glass Ionomer to Support an Indirect Ceramic Restoration: A Six-year Case Report

B Hammond • M Brackett • J Delash • W Brackett

Clinical Relevance

Using the technique of margin elevation, as described in this article, can often make a restoratively difficult situation more manageable and many times eliminate the need for more aggressive procedures that would ordinarily be required with traditional restorative techniques.

SUMMARY

This case report presents treatment of a mandibular second molar with an extensive proximal margin, which was finished on amalgam-stained dentin. A resin-modified glass ionomer for margin elevation and a lithium disilicate onlay were used to restore the tooth to proper form and function. The patient has been followed for six years and has had no complications during this period.

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INTRODUCTION

Removal of extensive caries that extends onto root surfaces and near the periodontal attachment often results in a preparation margin which is difficult to restore. Initial placement of an adhesive direct restoration in such areas to restore the missing root surface, resulting in a more accessible gingival margin for subsequent restoration of the remaining coronal structure, has been termed "margin elevation."[1-4] Margin elevation has the potential to simplify subsequent restorative procedures and to mitigate the need for surgical crown lengthening, leading to reduced treatment costs and shorter treatment duration.[5]

Because margins on root surfaces are on dentin, bonding is less optimal than if enamel were present,

regardless of the adhesive system chosen.[6,7] Although clinical studies of this type of restoration are difficult to conduct because margins are often obscured by gingival tissue, the extant literature indicates that either resin composite or resin-modified glass ionomer (RMGI) restorative materials are effective in margin elevation.[5,8-11]

When preparing teeth with extensive subgingival caries, it is often difficult to extend margins onto ideally mineralized dentin without impinging on the periodontal attachment and losing access for the subsequent restoration. When further extension would render the tooth nonrestorable without periodontal surgery, the clinician must decide whether or not to use compromised dentin as a restoration margin and proceed with margin elevation. Such dentin, although hard when instrumented, is probably in part demineralized, especially when extrinsic or amalgam stain is present.[12] Laboratory evidence indicates that both resin and RMGI demonstrate adhesion to demineralized dentin that is compromised relative to their adhesion to normal dentin.[13,14] However, there is lower percentage reduction in bond strength with RMGI systems compared with resin bonded systems, and the bond of RMGI to demineralized dentin remains stable over time.[14]

The following case describes the restoration of a left mandibular second molar with a fractured mesio-lingual cusp adjacent to a previous mesial-occlusal-buccal amalgam restoration that extended onto cervical dentin. This restoration had produced dark staining of the dentin, which could not be completely removed without extension into the periodontal attachment. Due to the likely demineralized status of this margin, it was elevated using an RMGI restorative material followed by preparation for a ceramic onlay, given the patient's preference for a tooth-colored restoration. A lithium disilicate (IPS e.max, Ivoclar Vivadent, Inc, Amherst, NY, USA) ceramic restoration was selected because of its combination of strength and effective resin bonding capability. Visual and radiographic evaluation of the restored tooth have been conducted over six years after placement of the restoration.

REPORT OF CASE

A 65-year-old female patient presented with the complaint of a fractured molar on her lower left side. Examination revealed the mesiolingual cusp of tooth No. 18 had fractured, leaving an adjacent mesial-occlusal-buccal amalgam restoration that extended apical to the cementoenamel junction (Figure 1). The patient was asymptomatic and had no periodontal or pulpal pathology, although it was observed that the existing mesial amalgam margin extended nearly to the sulcular depth. The patient wished to retain the tooth, and after the clinician explained the technical difficulties of effectively restoring the proximal margin with a conventional indirect restoration, she agreed to interproximal margin elevation. Because the distal cusps and marginal ridge were intact, a ceramic onlay was chosen for restoring the remainder of the missing coronal tooth structure.

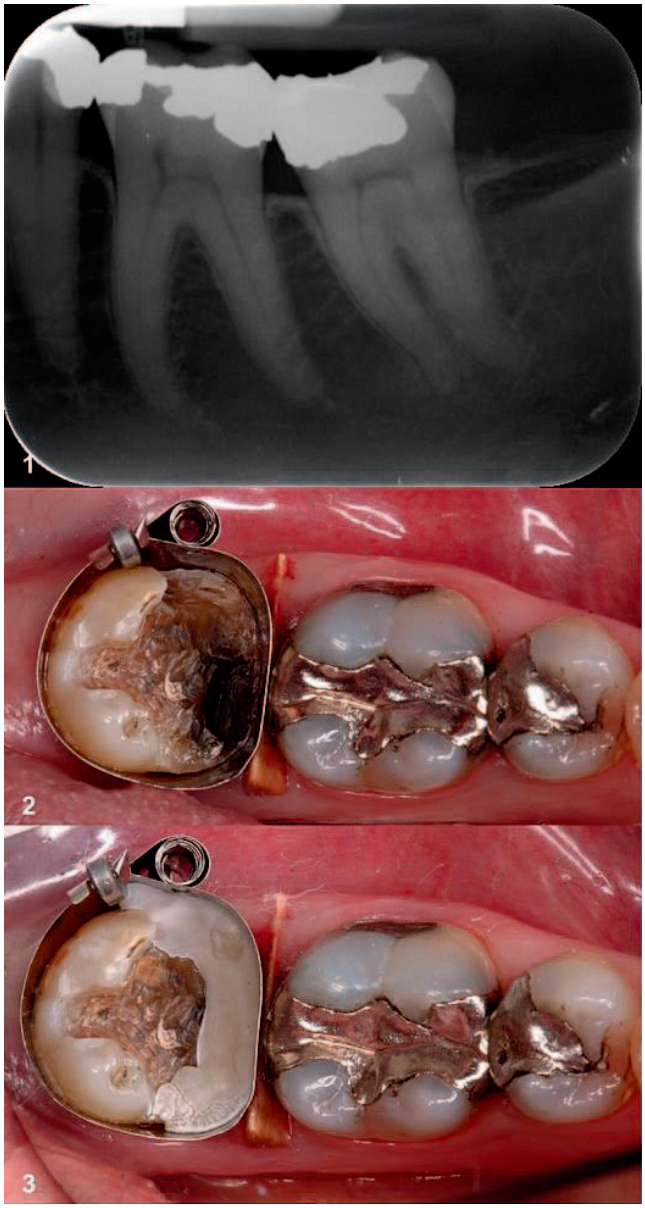

Figure 1. *Preoperative periapical view.*

Figure 2. *Preparation ready for resin-modified glass ionomer.*

Figure 3. *Resin-modified glass ionomer added.*

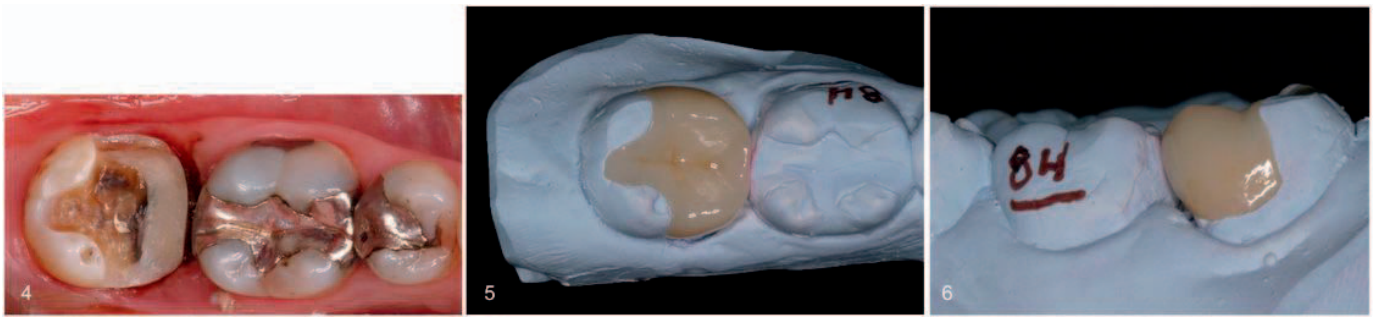

Figure 4. *Final preparation.*

Figure 5. *Onlay on solid cast, occlusal view.*

Figure 6. *Onlay on solid cast, buccal view.*

During tooth preparation, removal of the existing amalgam restoration revealed darkly stained dentin along the gingival margin. This dentin was removed to the extent that what remained was resistant to instrumentation with a slow speed No. 6 round bur but could not be completely removed without violating the periodontal attachment (Figure 2). Given the likelihood that the remaining dark dentin was partly demineralized, it was decided to raise the interproximal margin using an RMGI restorative material (Fuji II LC, GC America, Inc; Alsip, IL, USA).

An AutoMatrix band (Dentsply Sirona USA, Milford, DE, USA) was placed (Figure 2), the gingival floor of the preparation conditioned (Fuji Conditioner; GC America), and a capsule of the Fuji II LC restorative material was mixed and syringed into the mesial box up to the level of gingival papilla. Conditioning and mixing were carried out according to the manufacturer's instructions. The RMGI was cured with a light-emitting diode light (Valo, Ultradent Products, Inc. South Jordan, UT, USA) set to 1000 mW/cm^2 for 40 seconds (Figure 3). The matrix band was then removed and the RMGI layer again light cured for 30 seconds each from the buccal and lingual directions.

The preparation was refined (Figure 4) to meet the restoration requirements for a lithium disilicate (IPS e.max) ceramic onlay restoration. A full arch PVS impression (Extrude, Kerr Corporation, Orange, CA, USA) was taken along with an alginate impression of the opposing arch and a facebow record for mounting on a semiadjustable articulator. For the interim period, a self-curing bis-acryl provisional restoration (Protemp Plus, 3M ESPE. St Paul, MN, USA) was directly fabricated and cemented with non-eugenol–based temporary cement (Temp-Bond NE, Kerr Corporation). The lithium disilicate onlay (Figures 5 and 6) was fabricated by pressing using the lost wax technique and returned for delivery.

Approximately two weeks later, the patient was anesthetized and the provisional restoration and residual cement removed. The onlay was tried in for fit and the occlusion lightly verified and adjusted using a fine grit football diamond bur (No. 8379 0014, Komet USA. Rock Hill, SC, USA). The restoration was polished with diamond silicone polishing points (Dialite, Brasseler USA; Savannah, GA, USA) and steam-cleaned to remove any residual oral contaminants. The intaglio was then etched with 5% hydrofluoric acid (IPS Ceramic Etching Gel, Ivoclar Vivadent) for 20 seconds followed by thorough rinsing for 20 seconds. Next, in order to remove any residual porcelain precipitates produced by hydrofluoric acid etching, the intaglio of the restoration was lightly scrubbed using a microbrush with 37% phosphoric acid (Etch-37 with BAC, BISCO Dental Products, Schaumburg, IL, USA) for 30 seconds and rinsed. The restoration was thoroughly dried; silane (Silane, Ultradent Products) was applied and left in place for 60 seconds followed by thorough air drying for 30 seconds. The intaglio surface of the restoration was coated with a fifth-generation adhesive (Optibond Solo Plus, Kerr Corporation), which was air-thinned, after which the restoration was placed under a light protective barrier.

A nonlatex rubber dam (DermaDam, Ultradent Products) was placed over the patient's mandibular left quadrant using ligation of tooth No. 18 with unwaxed dental floss. The preparation, including the RMGI layer, was lightly cleaned using air particle abrasion at 30 psi with 50 μm aluminum oxide (Aluminum Oxide 50 Micron White, Danville Materials, San Ramon, CA, USA), rinsed, and lightly dried. The same 37% phosphoric acid was applied to

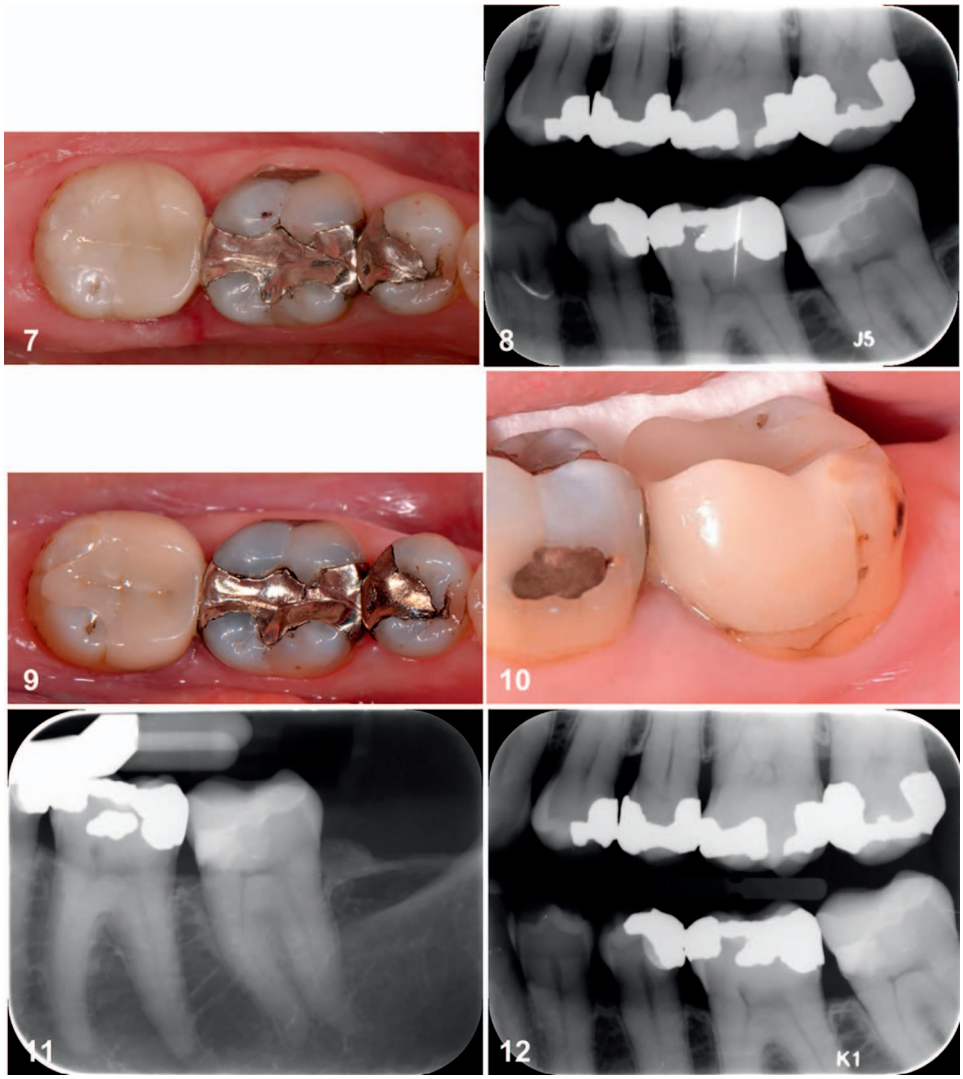

Figure 7. *Onlay after cementation and polishing.*

Figure 8. *Postoperative bitewing radiograph.*

Figure 9. *Six-year follow-up, occlusal view.*

Figure 10. *Six-year follow up, buccal view.*

Figure 11. *Six-year periapical radiograph.*

Figure 12. *Six-year bitewing radiograph.*

the enamel margins for 10 seconds, after which additional etchant was extended onto the dentin and RMGI base for an additional 10 seconds. The preparation was then thoroughly rinsed for 15 seconds and moisture evacuated using a high volume suction, leaving the preparation slightly moist.

Three coats of a fifth-generation adhesive (Optibond Solo Plus, Kerr Corporation) were applied successively followed by excess removal via high volume evacuation. After the third coat was thinned, the adhesive layer was thoroughly air-dried to remove excess solvent and ensure that no adhesive had pooled in the preparation that could have interfered with seating of the restoration. The dentin surfaces were visually inspected to ensure that a shiny surface was produced, indicating adequate resin infiltration. This adhesive layer was light polymerized for 20 seconds at 1000 mW/cm^2.

Clear resin composite cement (Nexus III, Kerr Corporation) was then applied to the intaglio surface of the restoration and syringed into the preparation and the onlay seated and stabilized while excess resin cement was removed using microbrushes (Kerr Applicators, Kerr Corporation) and unwaxed dental floss. The restoration was subsequently light polymerized at 1000 mW/cm^2 for 20 seconds each from the occlusal, buccal, and lingual surfaces. Glycerin gel was next applied to all margins to eliminate any oxygen inhibited layer and the restoration was again light polymerized for 20 seconds per surface.

The rubber dam was removed and occlusion checked again, followed by minor adjustment and polishing using the same fine grit diamond bur and polishing points. The final polish was completed with diamond polishing paste (Dialite Intra-Oral Polishing Paste, Brasseler USA) applied with a prophy cup (Figure 7). A post-cementation bitewing radiograph (Figure 8) was taken to verify complete seating and removal of all residual composite resin cement.

The restoration has been followed with annual visual exams and biennial bitewing and periapical radiographs for six years (Figures 9 through 12). The patient has remained asymptomatic with no evidence of caries, fracture of the tooth or restoration, periodontal inflammation, or pulpal pathology.

DISCUSSION

For the restoration described, the principal concern of the authors was the possibility for recurrent caries along the elevated mesial margin. Although evidence is limited to *in vitro* bond strength studies,[14,15] the authors considered an RMGI restorative material for this margin, which was placed on stained and partially demineralized dentin, to have the best prognosis. Although a conventional glass ionomer restorative material could have been used just as effectively in terms of its adhesion to dentin, an RMGI restorative material was selected for margin elevation over conventional glass ionomer due to the ability to develop a covalent bond between the RMGI and the resin cement used for adhesively bonding the ceramic onlay. Over the course of six years, there have been no recurrent caries, so this choice appears to be justified.

A second concern of the authors was that extension of the restoration into the gingival sulcus to this extent would provoke periodontal inflammation. The wish to minimize adverse periodontal effects led to the choice not to extend the proximal margin completely beyond the dark-stained root dentin, which likely would have placed the restoration margin into the periodontal attachment, and to the choice of margin elevation, which lessens the need for tissue retraction near the attachment associated with impression taking for an indirect restoration margin. The lack of bleeding on probing of periodontal tissue in the area of the restoration margin in this patient over six years tends to support these choices. The authors concur with other recent articles that recommend margin elevation more highly than periodontal crown extension surgery[5] for clinical situations like that of the patient reported here and believe that this strategy has been successful thus far in this patient.

The authors do not regret leaving the distal cusps, which appeared sufficiently strong and intact, and the performance of this relatively conservative restoration tends to support this choice. The selection of a lithium disilicate ceramic, rather than a stronger zirconia restoration, was based on the author's belief that optimal resin bonding to the ceramic was necessary to stabilize this restoration and was therefore a higher priority in this case than strength of the ceramic. Lithium disilicate, when of sufficient thickness and bonded with a resin cement, should withstand normal occlusal forces and provide good longevity. A gold onlay restoration would also have been an appropriate choice, but the patient declined a metal restoration.

Finally, the authors acknowledge that a direct cusp-replacement resin composite restoration could have been placed in this tooth after margin elevation. Although of higher initial cost, the ceramic restoration selected is more wear resistant and allowed the design of a more favorable restoration in terms of the proximal contact than was probably feasible with a direct restoration. Both alternatives were explained to the patient, who concurred with the choice of a ceramic restoration.

One consideration for future similar cases would be the placement of the RMGI in a thinner layer at the cervical margin. This modification would allow the emergence profile of the indirect restoration to begin at a slightly more cervical level, thereby providing a more convex contour of the restoration up to the contact zone. Providing this continuous convex surface would reduce the risk of an open gingival embrasure that could result in food entrapment possibly leading to recurrent caries if proper hygiene was not maintained. However, in this case, the restoration was successful, and the patient has not experienced food impaction or secondary decay.

SUMMARY

The elevation of an extensive interproximal amalgam-stained dentin margin with RMGI, done without periodontal surgery, followed by restoration with a lithium disilicate onlay as described in this clinical report, has been an effective treatment over six years. Providing conservative treatment whenever possible when restoring compromised teeth conforms to the main goals of restorative dentistry: conservation of tooth structure and supporting tissues and maintenance of pulpal vitality, all of which have been accomplished in this case.

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 College of Georgia at Augusta 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.

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Enamel Microabrasion and Dental Bleaching on Teeth Presenting Severe-pitted Enamel Fluorosis: A Case Report

D Sundfeld • CC Pavani • NIP Pini • LS Machado • TC Schott • RH Sundfeld

Clinical Relevance

The enamel microabrasion technique associated with dental bleaching is an excellent and successful clinical procedure for reestablishing the esthetics of a severe case of enamel fluorosis, eliminating the use of adhesive restorations.

SUMMARY

The present clinical case report describes the clinical steps of enamel microabrasion associated with dental bleaching to restore severely-pitted fluorosed teeth. The process of removing the fluorotic superficial stains started by using macroabrasion with a water-cooled fine tapered 3195 FF diamond bur. Rubber dam isolation of the operative field was used to remove the remaining enamel stains and superficial irregularities with the Opalustre microabrasive compound (6.6% hydrochloric acid associated with silicon carbide particles) followed by polishing using fluoridated paste and subsequent 2% neutral fluoride gel topical application. After one month, dental bleaching was performed using 10% carbamide peroxide in custom-formed acetate trays for two hours/day for 42 days. The association of enamel microabrasion with dental bleaching was effective for reestablishing the dental esthetics of a patient with severe dental fluorosis.

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INTRODUCTION

Enamel alterations/stains are a multifactorial clinical condition that, in most cases, negatively affects the beauty and attractiveness of a smile.

Early- and post-stage caries (white spots), white spots around orthodontic brackets, scratches after orthodontic bracket removal, amelogenesis imperfecta, dental fluorosis, enamel hypoplasia, and molar incisive hypomineralization are the most frequent causes of those physical and esthetic enamel alterations.[1-8] Despite the distinct etiology, enamel microabrasion, dental bleaching, resin infiltration, and direct and/or indirect restorations are frequently adopted for reestablishing the esthetics of the affected teeth.

Dental fluorosis is an undesirable mineralization defect of dental enamel and is recognized as a side effect of the excessive and chronic ingestion of fluoride during amelogenesis, with the severity of the condition linked to "fluoride dose and the timing and duration of fluoride exposure."[9] Thus, with increased fluoride intake, the enamel defects and severity of fluorosis increases.[10,11] The most common fluoride sources are tap and well water, diet (children's cereals, chocolate-flavored milk, biscuits), bottled water and beverages (soft drinks, juices, and teas), salt, dietary supplements, and fluoride-containing dental products (dentifrices, mouth rinses, varnishes).[9,12-18] Teeth diagnosed with dental fluorosis present "white opaque areas or discolorations ranging from yellow to dark brown, in combination with porosities on the enamel surface" located on homologous teeth.[19]

Depending on the severity, dental fluorosis has a direct negative impact on the social quality of life.[15,20] Despite the possible negative consequences on the appearance of teeth, fluoridated water and other fluoride sources, such as fluoridated dentifrices, are recognized as important and effective public health strategies for preventing and reducing the incidence of caries.[9,21-24]

The esthetic treatment of enamel fluorosis is closely related to its degree of severity. Patients presenting with mild or very mild fluorosis, a clinical condition that does not always affect the quality of life,[20,25] may be submitted to enamel microabrasion and/or dental bleaching, treatments that remove and/or camouflage the white spots, respectively. On the other hand, moderate and severe enamel fluorosis have a direct impact on a patient's quality of life, and esthetic/restorative treatment (eg, ceramic restorations) is necessary.

Therefore, the aim of the present clinical case report is to demonstrate the esthetic recovery of a severe case of enamel fluorosis submitted to enamel microabrasion followed by dental bleaching.

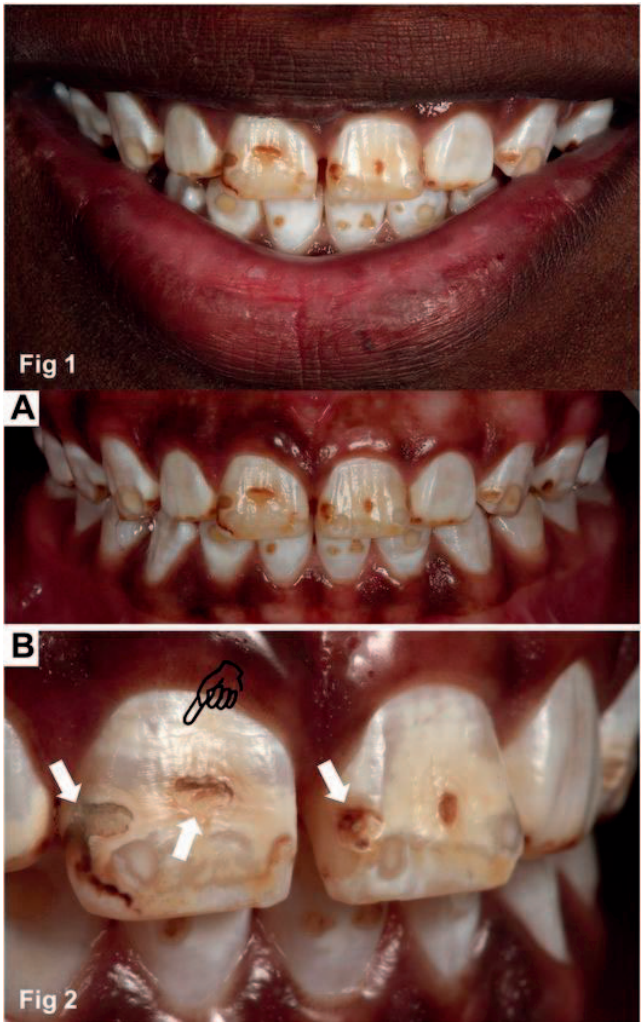


Figure 1. *A 17-year-old boy presenting with white and brown stains with a hard texture located on the facial and lingual surfaces of all teeth. Pitted areas are also found on the facial surface of anterior teeth.*

Figure 2. *Intrafacial view of the affected teeth (A). Note the pitted areas on the facial surfaces of the central maxillary incisors (arrows) and the accentuated perikymata (pointer) (B).*

CASE REPORT

A 17-year-old boy with white and brown colorations with a hard texture and pitted eroded areas (Figures 1 and 2) presented to Araçatuba Dental School/UNESP seeking esthetic treatment. After taking an accurate medical history and performing clinical analysis, a severe case of enamel fluorosis was diagnosed. Clinicians explained the treatment options to both parents and patient, and the enamel microabrasion technique was chosen to remove the stains and pitted areas on the facial surfaces of the upper and lower incisors, canines, and premolars.

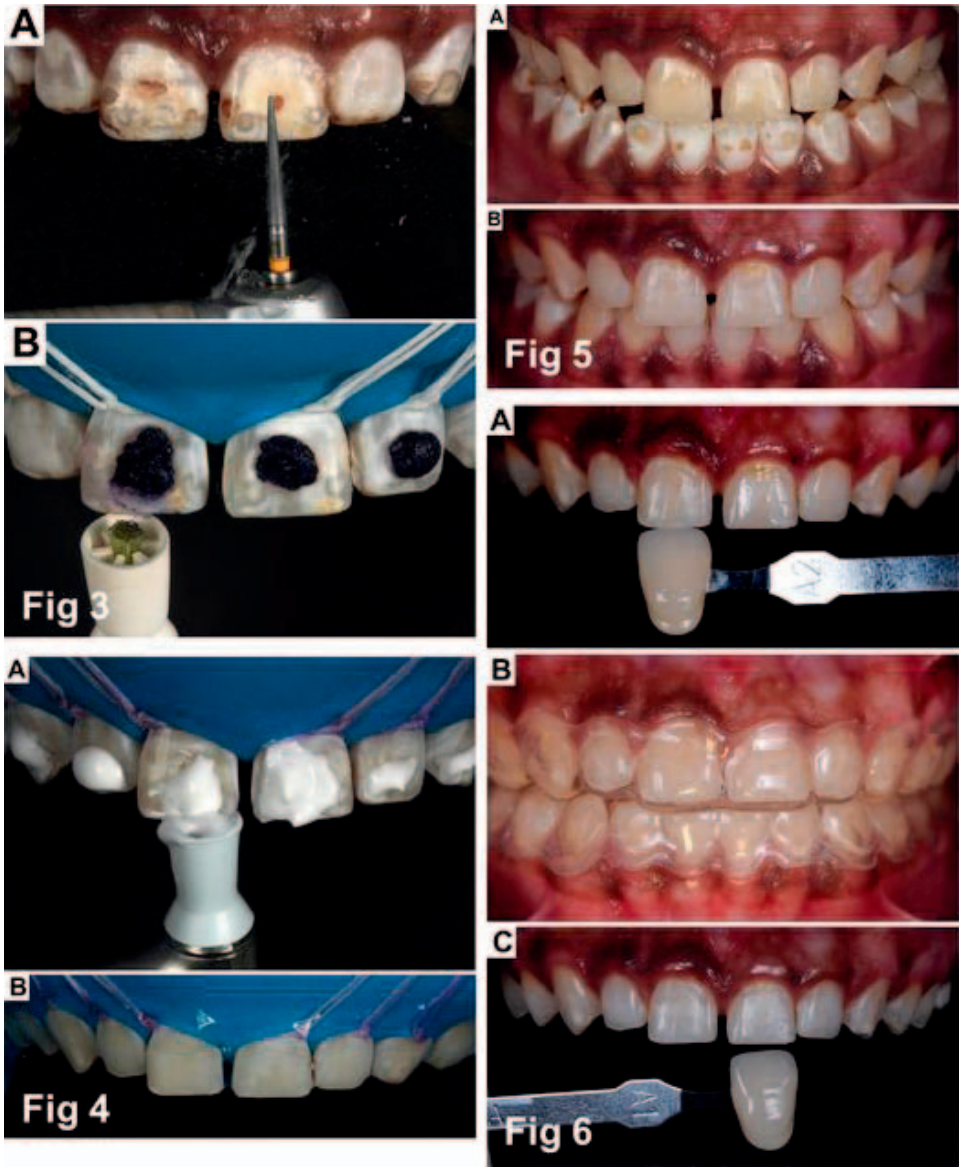


Figure 3. *Macroabrasion using a tapered fine diamond bur (No. 3195 FF, KG Sorensen) with a high-speed hand piece in the presence of water (A). Under rubber dam isolation, application of Opalustre (Ultradent; 6.6% hydrochloric acid associated with silica carbide particles) using a specific rubber cup (OpalCups, Ultradent) (B).*

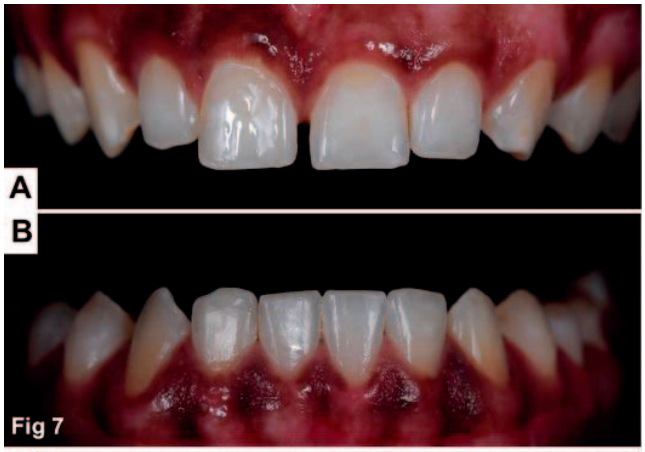
Figure 4. *Polishing with fluoridated paste using a low-speed hand piece (A) followed by the application of 2% neutral-pH sodium fluoride gel for 4 minutes (B).*

Figure 5. *Frontal view right after the first macro/microabrasion session of the upper arch (A). Final view after macro/microabrasion of the lower arch (B).*

Figure 6. *Assessing tooth shade using a Vitapan Classical Shade Guide (A). Acetate custom trays positioned on the upper and lower arches for home-bleaching treatment (B). Evaluation of final color achieved after dental bleaching (C).*

After dental prophylaxis with pumice and water, the superficial layer of the stained enamel on the facial surfaces of the upper and lower incisors, canines, and premolars was removed (macroreduction) using a fine-tapered bur (3195 FF, 30 μm; KG Sorensen, Barueri, SP, Brazil) under copious water irrigation attached in a high-speed hand piece (Figure 3A).

After protecting the gingival tissues with petroleum jelly, the operative field was isolated with a rubber dam to protect the gingival tissues from the microabrasive compound. The patient, assistants,



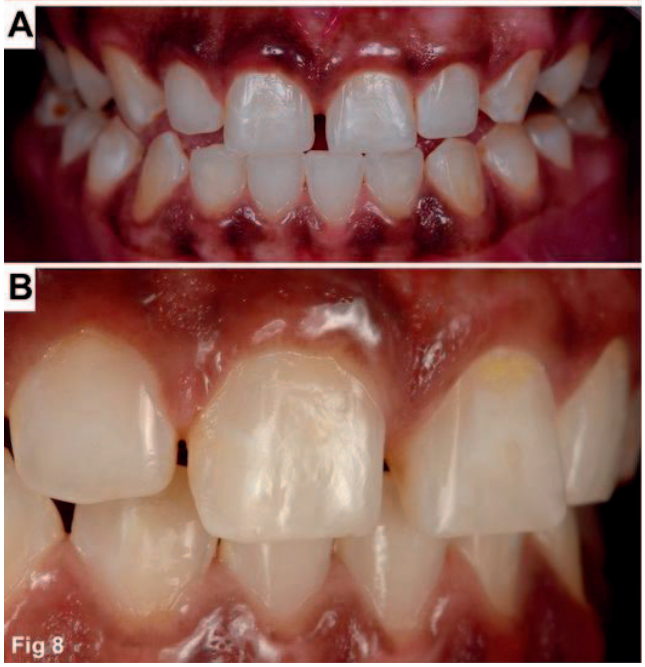


Figure 7. *Intrafacial view of upper (A) and lower (B) arches after enamel microabrasion treatment associated with dental bleaching.*

Figure 8. *Intrafacial view after accomplishing dental bleaching (A). Side view of maxillary incisors showing the complete removal of the pitted areas and the subsequent healthy, shiny and polished enamel surface acquired after enamel microabrasion (B).*

and operator wore individual protective equipment and eye protection during all the clinical procedures. The enamel microabrasive product (Opalustre, Ultradent, South Jordan, UT, USA) was used to remove the remaining stains using a specially designed rubber cup (OpalCups, Ultradent) in a low-speed hand piece under firm hand pressure (Figure 3B). The microabrasive compound was applied three times for one minute each on the facial surface of the stained teeth, with five seconds on each tooth for a total of one minute, with water rinsing between each application. After polishing the

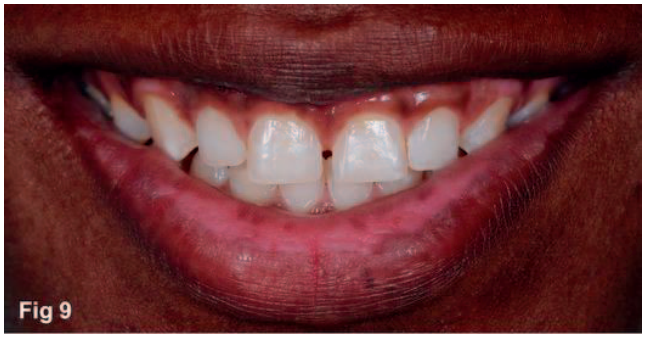


Figure 9. *Extraoral view after enamel microabrasion treatment associated with dental bleaching. Note the more pleasant and harmonic smile.*

abraded enamel surface with fluoridated paste (Herjos, Vigodent, Rio de Janeiro, RJ, Brazil) (Figure 4A), a neutral 2% sodium fluoride gel (Apothicário Manipulation Pharmacy, Araçatuba, SP, Brazil) was applied for four minutes (Figure 4B). Figure 5 shows the enamel appearance after enamel microabrasion on the upper and lower arches.

After one month, dental bleaching was performed using 10% carbamide peroxide (Opalescence, Ultradent) (Figure 6). Alginate impressions of the upper and lower arches were made. Stone models were poured and used to fabricate the custom acetate bleaching trays. The patient was instructed to place a small drop of the bleaching gel into each tooth section of the acetate trays and advised to wear the bleaching product for two hours/day. Six tubes of the bleaching gel were used during all the bleaching treatments.

The effectiveness of dental bleaching (dental color change) was evaluated before (Figure 6A) and after (Figure 6C) the bleaching treatment in the upper and lower incisors and canines using a visual method (Vitapan Classical Shade Guide; Vita Zahnfabrik, Bad Sackingen, Germany), with the results indicating a color improvement from A2 to A1.

Assessment of dental sensitivity was performed at baseline; at days 7, 14, 21, 28, 35, and 42 of the bleaching treatment; and 14 days after completion of the bleaching treatment. No gingival irritation or sensitivity was observed and the patient was satisfied with the enamel microabrasion and bleaching treatment. Figures 7 through 9 depict the final appearance (at the four-month follow-up) after enamel microabrasion and dental bleaching.

DISCUSSION

In the past, removal of sound and affected dental tissues, followed by resin composite restorations,

was indicated as a dental treatment for fluorosis. However, the present clinical case report demonstrated that a severe case of enamel fluorosis may be successfully treated with enamel microabrasion and dental bleaching.

The scientific literature shows that an excessive ingestion of fluoride during amelogenesis affects ameloblast function and the enamel matrix, inducing changes in the developing enamel. Clinical changes in the enamel vary from chalky white opaque areas, which result from enamel subsurface hypomineralization (which increases with fluoride exposure, resulting in different levels of severity)[26] because the enamel matrix secreted during fluoride exposure fails to mineralize, to pits, grooves, and post-eruption staining in more severe cases,[10,27] making enamel more prone to fracture and wear.[28] During clinical evaluation, clinicians must be aware that the presence of white areas reveals the existence of underlying hypomineralization and that enamel fluorosis symmetrically affects groups of homologous teeth.[2]

As depicted in Figures 1 and 2, the present case report depicts a severe case of enamel fluorosis showing deep pits on the facial surface. The parents and patient reported that the pits were not found right after eruption but developed after eruption. It may be hypothesized that mechanical damage (trauma), such as drinking soft drinks in glass bottles positioned in the upper facial surface or teeth brushing, removed the surface layer that covered the hypomineralized subsurface enamel, exposing the softer enamel tissues and turning them into pits. Pitting severity is determined by the degree of enamel porosity at the time of eruption and the exposure of the tooth surfaces to masticatory forces or by other substances in the mouth,[2,29] causing secondary modifications to the enamel texture (pitting and fissures).[2]

As seen in Figure 2B, the accentuated perikymata is likely associated with chronic exposure to fluoride, which is linked to the first signs of enamel fluorosis.[10] This finding is compatible with the patient's medical/dental history, as both parents and patient reported the use of a very large amount of flavored dentifrice (1100 μg F/g; Tandy, Kolynos do Brasil, São Paulo, SP, Brazil)[30,31] on the toothbrush, which the patient used to swallow between the ages of six and eight years. Also, the parents and patient reported the use of fluoridated mouthrinses at school after lunch between the ages of three and five years, which the patient remembered occasionally swallowing. The patient also reported the daily consumption of soft drinks that contained fluoride,[13] which is associated with moderate and severe forms of fluorosis.[18]

The critical period for the increased risk of developing dental fluorosis is between three and six years of age, as fluorosis is related to periods of permanent dentition development.[32,33] Hong and colleagues[34] considered up to 48 months the critical age for fluorosis development in permanent maxillary incisors, with the first two years of life the most critical period (higher risk). The combined fluoride intake from dentifrice, mouthrinses, and soft drinks may have led to increased levels of fluoride, which was incorporated into the dental hard tissues (fluorotic teeth presents higher fluoride content than sound teeth),[35,36] directly influencing the quality of amelogenesis. The city's tap water, which the patient used during infancy, was not considered the main factor involved in the fluorotic enamel because the city's (Penápolis, SP, Brazil) fluoride levels were within the optimum risk-benefit range.[16]

The lack of supervision from the parents and school staff in regards to advising the patient to spit out toothpaste and mouthrinse likely directly contributed to the development of dental fluorosis. As the intake of fluoride from dentifrices is directly related to the amount of toothpaste used, frequency of brushing, age, and especially flavored dentifrices,[33,37-41] it is necessary for parents/caregivers to instruct and monitor the proper use of fluoridated dentifrices, while also telling the children to not swallow the dentifrice. Also, it is important for parents/caregivers to use a pea-sized amount of dentifrice during toothbrushing, which has been recommended by dentists and reduces the risk of dental fluorosis.[39,40,42] Moreover, toothbrushing should be encouraged after meals to decrease the bioavailability of fluoride.[43]

Despite the lower or nonexistent negative impact of mild and very mild enamel fluorosis,[20,44,45] the psychosocial impact of severe fluorosis on the patient's quality of life is devastating.[15,20,41] Young people can make negative value judgments about other young people based on the smile's esthetic appearance and on misconceptions that those suffering from fluorosis do not care about their teeth.[46] During the medical exam, the patient reported being unhappy with and shamed by his smile; he also reported applying liquid paper on the facial surfaces of his anterior teeth to hide the fluorotic enamel surface. De Castilho and others[47] posited that "The clinical characteristics of fluorosis lead to shy, sad, and quiet children who cover their faces with their

hands to express happiness, smile with closed lips, do not participate in school social activities, and avoid talking with their classmates and smiling due to the shame of their dental appearance." The patient reported all of those undesirable situations.

Enamel microabrasion is usually performed on mild to moderately fluorosed teeth, with a clinical success that is more predictable because the stains are more superficial. However, enamel microabrasion only slightly improves or may even be ineffective on the appearance of moderate to severely fluorosed teeth because enamel staining and opacities can penetrate to deeper enamel levels.[48] Thus, the efficacy of the enamel microabrasion technique is closely related to the severity of dental fluorosis.[48] On the other hand, the present clinical case report demonstrated the efficacy of this clinical technique on severely fluorosed teeth. At the beginning of the treatment, the authors were not sure whether the enamel microabrasion would be enough to improve esthetics. The patient and parents were aware that a conservative attempt at correcting the fluorosis would be made (enamel microabrasion) to avoid an excessive loss of tooth structure before adopting a more invasive approach (stain removal with diamond burs followed by resin composite veneer restorations).

Macroabrasion is a simple, low-cost technique that is performed using a fine-tapered diamond bur to lightly abrade and remove superficial stains and enamel irregularities (Figure 3A).[1] This procedure reduces the time needed for removing the fluorotic stains and the amount of microabrasive material to be used, especially when faced with more pronounced and deep intrinsic stains, as in the case of severe enamel fluorosis. Application of the fine-tapered bur is optional, but if it is not used will lead to a longer treatment time and more than three applications of the microabrasive product. Later, the stain removal was complemented and the enamel surface was smoothed using a microabrasive product (Opalustre, Ultradent), which is composed of 6.6% hydrochloric acid associated with abrasive silicon carbide particles, a product that presents an excellent clinical performance in smoothing the enamel irregularities/scratches left by the fine-tapered diamond bur.[5]

After enamel microabrasion, teeth can acquire a yellowish appearance due to the reduction in enamel thickness, which makes the underlying dentin more apparent.[1] Therefore, dental bleaching was adopted in this clinical case to produce a lighter tooth structure that minimized the contrast between the areas of healthy and stained enamel.[49] Previous studies reported that the association of enamel microabrasion and dental bleaching provided better and more satisfying esthetic outcomes compared with enamel microabrasion alone.[19,49] Additionally, the patient in the current case study reported no adverse side effects, such as dental sensitivity or gingival discomfort.

It is important to note that enamel microabrasion is a low-cost, easy, and conservative technique that permanently removes intrinsic stains and has proven clinical efficacy and longevity.[1,3-5,7,8] The simultaneous erosive (hydrochloric acid) and abrasive (silicon carbide particles) action of the microabrasive compound produces the "abrosion effect", a condition that densely compacts the prism-free layer on the enamel surface.[50] Clinically, the consequence is a smooth, shiny, lustrous, and healthy enamel surface (Figures 7 through 9) that maintains its esthetic quality over time.

CONCLUSIONS

Severe enamel fluorosis is a challenging and difficult case for dentists and represents a negative effect on the patient's quality of life. A conservative treatment does not always correct the esthetics of the affected teeth, and sometimes a more invasive restorative treatment is necessary. The present clinical case report described enamel microabrasion and dental bleaching as an effective and safe technique for treating a severe case of enamel fluorosis.

Regulatory Statement

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

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Long-term Follow-up of Complicated Crown Fracture With Fragment Reattachment: Two Case Reports

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Clinical Relevance

Complicated crown fracture in permanent teeth may cause restorative problems with an unfavorable prognosis. The fragment reattachment technique is the most conservative treatment option.

SUMMARY

Two cases of complicated crown fracture of the maxillary incisors were restored using the fragment reattachment technique. Root canal treatment was performed, and the fractured fragment was bonded to the tooth structure using a dentin adhesive system and a flowable composite resin, followed by the insertion of a fiber post using dual-cured resin cement. Reattached fragments have shown reliable prognosis without inflammatory signs around bonded junctions after long-term follow-up.

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INTRODUCTION

Approximately 25% of all school-aged children and 33% of adults experience trauma to the permanent dentition.[1] Complicated crown fracture, which means a crown fracture accompanied by pulp exposure, accounts for 2-13% of all traumatic dental injuries.[2-4] The most commonly involved region of complicated crown fracture is the maxillary incisors,[3-5] which presents many challenges to clinicians. Treatment planning and long-term prognosis depend on various factors such as fracture line

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location, biologic width, root development, alveolar bone fracture, soft-tissue injury, occlusion, and esthetics.[2,6] The fragment reattachment technique, first reported in 1964, includes root canal treatment followed by restoration with a cast post and core.[7] Since the advent of adhesive dentistry, the total-etch technique has been applied in fragment reattachment for reinforcement and the retention of fractured tooth fragments.[8,9]

The fragment reattachment technique can preserve the original translucency, morphologic contours, and natural surface textures compared with composite restoration and full-coverage crowns.[10] It is the most conservative and least time-consuming treatment for complicated crown fractures. Previous reports presented successful management of subgingival crown fractures via the adhesive technique and fiber post with a follow-up period of 12 months to four years.[11,12]

Other options to restore complicated crown fractures are surgical crown lengthening, forced eruption, and surgical extrusion.[13,14] Surgical crown lengthening involves osseous and gingival contouring including the adjacent teeth, thereby compromising esthetics and function.[15] Forced eruption requires a treatment period over three months and additional periodontal intervention.[14,16] Surgical extrusion accompanies full-coverage crowns and carries the complications of inflammatory or replacement root resorption.[13,17] Moreover, those three methods cause root length shortening. There have been no randomized controlled trials on the forced eruption and surgical extrusion, yet clinically successful results were reported up to 12-24 months.[13,17,18] The objective of this report is to present two cases of complicated crown fracture using the fragment reattachment method with intracanal reinforcement via a fiber post.

CLINICAL TECHNIQUE REPORT

Case 1

A 15-year-old girl fell at the stairs and visited our emergency center. An oral and maxillofacial surgeon sutured her lacerated upper lip and referred her to the department of conservative dentistry for the treatment of fractured teeth. Complicated crown fractures were visible in the maxillary central incisors (#8, #9), and uncomplicated crown fractures were observed in the maxillary right and left lateral incisors (#7, #10) (Figure 1A,B). The fractured fragments of #8 and #9 were not completely separated from the remaining tooth and exhibited moderate mobility. The fracture line of #8 was supragingival on the labial side and subgingival on the palatal side, whereas that of #9 was located subgingivally on the labial and palatal side. A periapical radiograph revealed obvious fracture lines on #8 and #9 (Figure 1C). For both incisors, root canal treatment followed by fragment reattachment with a fiber post was planned.

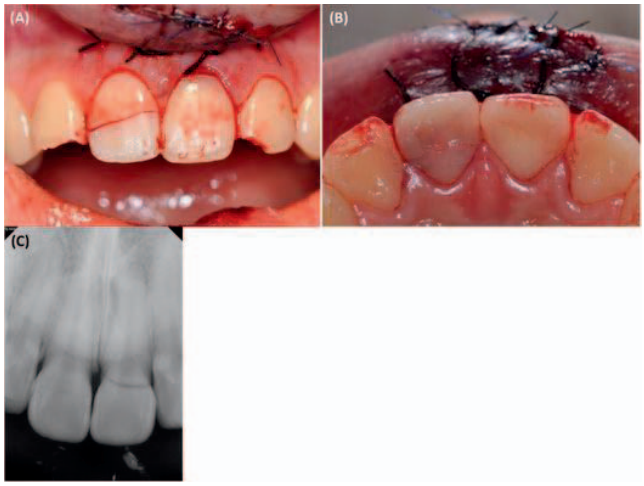

Figure 1. *(A, B) Preoperative photographs of fractured teeth (#8, #9) with complicated crown fractures and #7 and #10 with uncomplicated crown fractures. (C) Intraoral radiograph of fractured teeth.*

At the second visit (one week after the first visit), fractured fragments were removed from #8 and #9. The fragment of #8 was divided into two pieces, and discoloration was observed on the fragment of #9 (Figure 2A,B). On the day the fragment was removed, a single-visit root canal treatment was performed for each central incisor (Figure 2C). The two fractured fragments of #8 were bonded with dual-cure resin cement (Duo-Link, BISCO Inc, Schaumburg, IL, USA) and acetone-based etch & rinse adhesive (One-Step Plus, BISCO Inc). An access hole was made on the palatal side of each fragment to create room for post placement and vent space for the cement spillway (Figure 2D). A slight bevel with 0.5-mm thickness was prepared at the supragingival margin of the fractured teeth and all fractured margins of the fragments. Retention grooves with 0.5-mm depth were made within the fractured fragments (Figure 2E,F). The fractured surface was exposed using flap elevation under local anesthesia. A fiber post (DT-Light Post, BISCO Inc) of the proper size was chosen and adapted after the post space preparation. The fractured surfaces on the fragments, post space, and fractured teeth were treated with 35% phosphoric acid etchant (Select HV Etch, BISCO Inc), and each fragment was bonded to

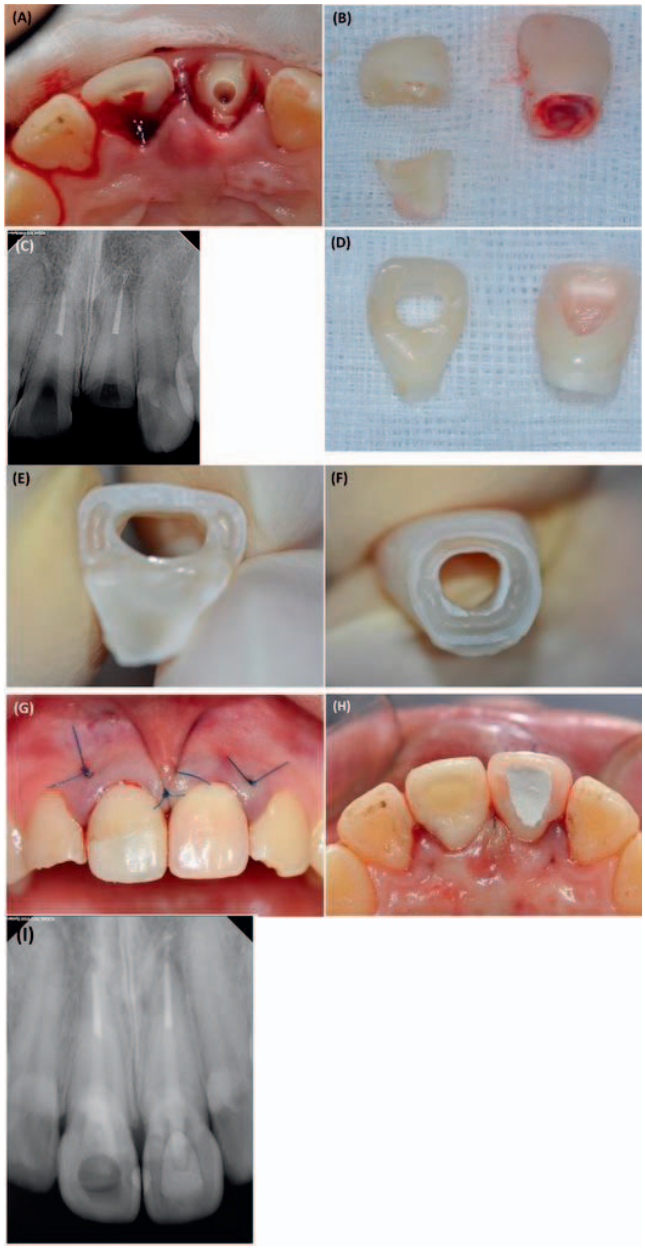

Figure 2. *(A) Photograph of fractured teeth after removal of the fractured fragments. (B) Fracture fragment of tooth #8 (left) and #9 (right). (C) Intraoral radiograph after root canal treatment. (D) Two segments of fractured fragment of #8 were bonded, and an access hole was prepared on the palatal side of the fractured fragment of #8 (left) and #9 (right). (E, F) Internal dentin groove formed on the fractured fragment of #8 and #9, respectively. (G, H) Reattachment and suturing was performed. (I) Periapical radiograph after reattachment.*

the tooth fractured surface with flowable composite (G-aenial Flo, GC, Tokyo, Japan) and One-Step Plus. Next, the fiber post was cemented using Duo-Link. After careful removal of the excess cement, the flap was sutured with 4-0 nylon silk (Figure 2G-I). The access hole of #8 was restored immediately with

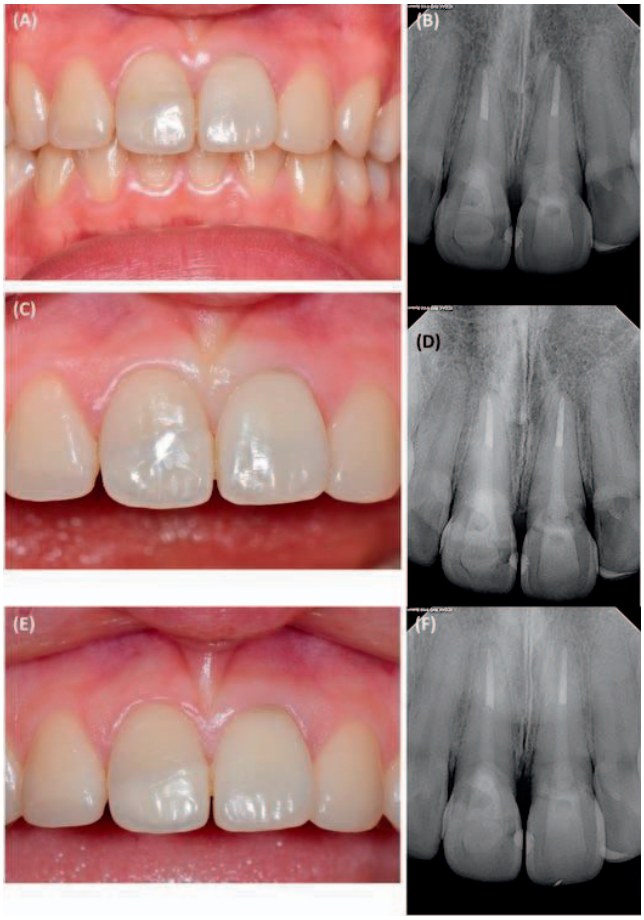

Figure 3. *(A) One-year follow-up photograph and (B) intraoral radiograph. (C) Two-year follow-up photograph and (D) intraoral radiograph. (E) Four-year follow-up photograph and (F) intraoral radiograph.*

composite resin (Estelite Sigma Quick, Tokuyama Dental, Yamaguchi, Japan) and One-Step Plus, whereas that of #9 was filled with temporary filling material (Caviton, GC) for nonvital bleaching (Figure 2H) performed with sodium perborate for two weeks. Two weeks after the reattachment, the wound area exhibited a normal healing appearance, and the colors of teeth #8 and #9 matched the surrounding dentition. The access hole of #9 was restored using the same materials as #8. The vitality of #7 and #10 remained after suture removal, and each was restored with Estelite Sigma Quick and One-Step Plus. One year later, the function and esthetics of the teeth and periodontal tissue were good, and the patient was asymptomatic (Figure 3A). Apical healing of #8 and #9 was examined on a periapical radiograph (Figure 3B). In a visit two years after treatment, the function and esthetics remained good, and there were no pathologic findings on a periapical radiograph (Figure 3C,D).

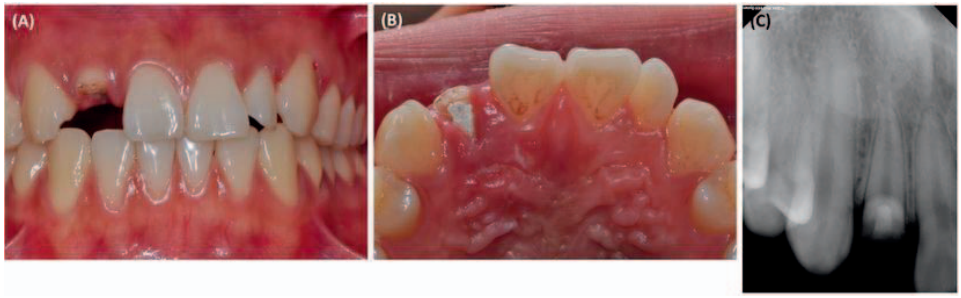

Figure 4. *(A, B) Preoperative photographs of fractured tooth #7 with a complicated crown-root fracture. (C) Intraoral radiograph of the fractured tooth.*

Follow-up checks after four years showed no sign of weakened bonding strength or inflammatory root resorption (Figure 3E,F).

Case 2

A 23-year-old man visited the department of conservative dentistry with the chief complaint of a broken upper anterior tooth. Clinical and radiographic examinations revealed a complicated crown fracture of the maxillary right lateral incisor (#7) (Figure 4A-C). He had fallen down in the street prior to visiting the clinic. A pulp extirpation had been performed on his tooth immediately after the trauma at an emergency center. The patient brought his fractured fragment stored in saline. Root canal treatment followed by fragment reattachment with a fiber post was planned.

After the root canal treatment was completed, the post space was prepared for a DT-Light Post (Figure 5A). An access hole was prepared in the palatal surface of the fractured fragment to create a cement spillway, and a retention groove was made within the dentin to enhance the retention (Figure 5B). Flap elevation was performed on the labial and palatal side under local anesthesia (Figure 5C), and an osteotomy was performed of the palatal side to obtain sufficient biologic width. The fracture surfaces on the fragments, fractured teeth, and post space were treated with a Select HV Etch, whereas the fragment was bonded to the fractured surface with G-aenial Flo and One-Step Plus. Thereafter, the post was cemented using Duo-Link and One-Step Plus, the access hole was restored with composite resin (Charisma Classic, Heraeus Kulzer GmbH, Hanau, Germany), and the flap was sutured with 4-0 nylon (Figure 5D-F). At the four-month follow-up visit, clinical and radiographic examinations showed stable reattachment and periodontal condition (Figure 6A-C). At the five-year follow-up visit, slight discoloration was observed at the cervical area (Figure 6D). However, the clinical and radiographic examinations showed normal function and shape without

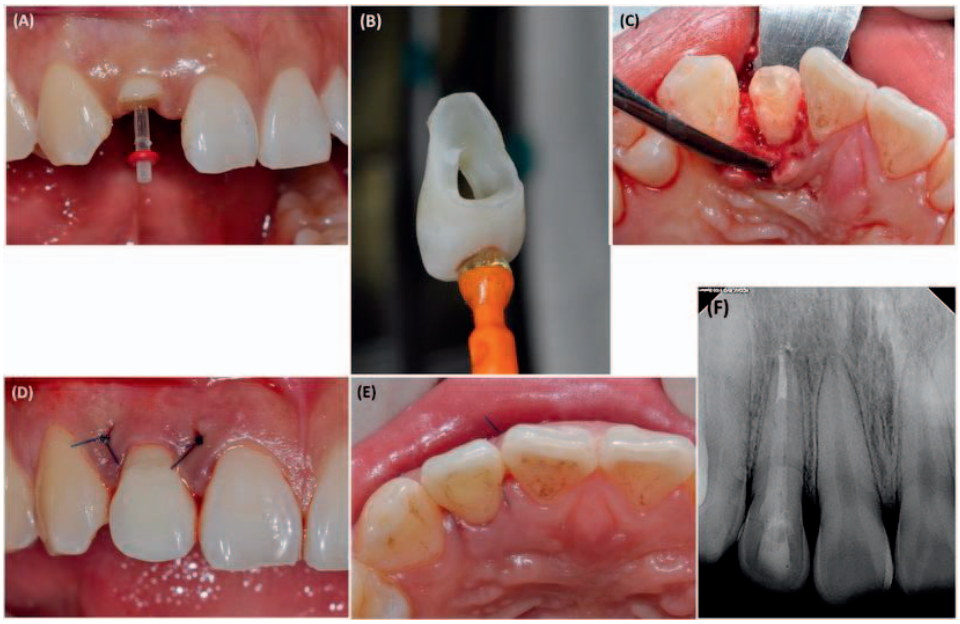

Figure 5. *(A) A fiber post was selected within the post space of tooth #7 after root canal treatment. (B) An access hole was prepared on the palatal surface of the fractured fragment. (C) A palatal flap was raised, and an osteotomy was performed on the palatal side. (D, E) After reattachment and flap suturing and (F) intraoral radiograph after reattachment.*

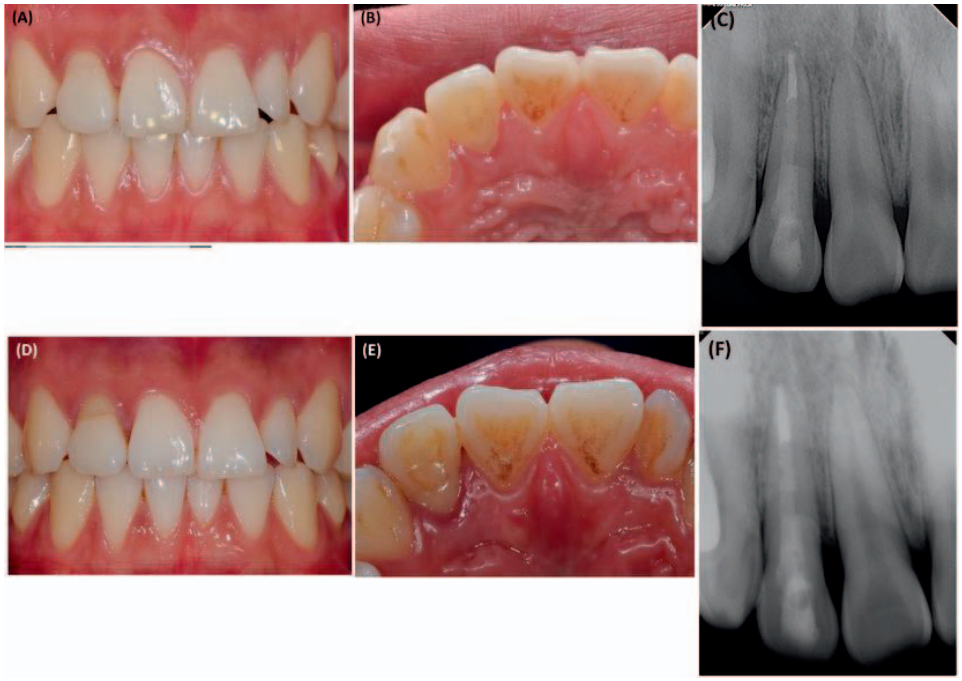

Figure 6. *(A, B) Four-month follow-up photographs and (C) intraoral radiograph. (D, E) Five-year follow-up photographs and (F) intraoral radiograph.*

weakened bonding or inflammatory root resorption (Figure 6D-F).

DISCUSSION

In both cases, use of the fragment reattachment technique restored the original tooth contour with no signs of gingival inflammation. Even if the reattached fragment would be detached, other treatment methods can be used. Reattachment is the most conservative approach especially in young patients for managing complicated crown fracture.[10]

A fiber post, dual-cure resin cement, and composite core material were used to increase retention and provide a mono-block effect,[11,12,19] which is a gap-free, mechanically homogeneous mass in the root canal space that consists of different bondable materials with similar elastic modulus.[19] A fiber post has some advantages over a cast metal post, such as a similar elastic modulus to that of dentin and high durability.[20,21] It also presents relatively uniform stress distribution to the tooth due to its similar elastic modulus to that of dentin, thereby yielding protection against root fracture.[22,23] Because the establishment of stable adhesion is mandatory for fiber post cementation, Duo-Link and One-Step Plus were used for post cementation. Generally, post space has unfavorable condition for adhesion due to tremendous configuration factor and limited light transmission.[23] One-Step Plus is a total-etch acetone-based dentin adhesive with low acidity (pH 4.6) that shows favorable compatibility with the self-cure composite.[24] Some single-step self-etch adhesives or two-step total-etch adhesives with high acidities showed incompatibility with self-cure composite.[25-26] In addition, the possible contamination during solvent evaporation at the bonded interfaces may be minimized because acetone has a higher evaporation pressure than ethanol or water.[27] G-aenial Flo, which was used to adhere the fractured fragments, is a flowable composite with higher viscosity than other flowable composites. Its use enables the minimizing of excess after fragment adaptation and facilitates excess removal.

The fracture lines in both cases demonstrated an oblique pattern from the labial to palatal side that was unfavorable to bear occlusal forces applied in the labial direction.[28] Nevertheless, both cases showed stable reattachment up to four and five years. The stable reattachment was mainly attributed to proper adhesive procedures.[23,29]

The technique used to increase retention and resistance for reattachment of a fractured fragment includes enamel beveling, a V-shaped internal enamel groove, an internal dentin groove, an external chamfer, and overcontouring.[30] According to Demarco and others, fracture resistance of the reattached coronal tooth structure was increased when a bevel was prepared.[31] De Santis and others

reported that fracture resistance of a reattached bovine incisor was increased when a circumferential chamfer was prepared and filled with composite resin compared with a simple reattachment method.[32] Reis and others demonstrated that overcontouring and an internal dentin groove recovered 97.2% and 90.5% of the fracture resistance for the intact tooth, respectively, whereas simple reattachment recovered only 37.1% and the buccal chamfer recovered 60.6%.[29] Accordingly, an internal dentin groove preparation was used in both cases and enamel beveling was used in case 1.

The palatal fracture margin was located below alveolar bone in case 2, where surgical crown lengthening and an osteotomy were performed to ensure sufficient biologic width. However, such a surgical intervention was not done in case 1, which included a subgingival fracture on the palatal side of tooth #8 and on the labial and palatal sides of tooth #9. Unlike case 2, the fracture lines were located at the same level as the alveolar crest in case 1. When subgingivally placed composite resins received sufficient polymerization and optimal finishing, polishing did not adversely affect the gingiva.[33-35] During follow-up, no gingival inflammation or alveolar bone loss was noted. Polished, planed, and nonirritating subgingival margins in combination with distinct oral hygiene contributed to the prolonged good prognosis in the case of subgingival fracture.[36] This suggests that proper adhesion enables the maintenance of a sound periodontal condition.

CONCLUSIONS

Here we demonstrated fragment reattachment in two cases of complicated crown fracture with esthetic and biocompatible restoration. By using the materials available today in combination with an appropriate technique, predictable esthetic outcomes of crown attachment can be achieved. The use of a fiber post along with adhesive technology may be a sound restorative alternative and less invasive procedure than full-crown coverage.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Kyung Hee University School of Dentistry, Seoul, Korea.

Conflict of Interest

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

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Clinical Research

Six-month Follow-up of the Effect of Nonvital Bleaching on IL-1β and RANK-L: A Randomized Clinical Trial

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Clinical Relevance

The "walking bleach" technique induces an increase in IL-1β and RANKL production in periodontal tissues, which persists for six months after treatment.

SUMMARY

Objectives: It has been reported that bleaching generates an increase in the activity of osteoclasts *in vitro*. We quantified the RANK-L and IL-1β biomarkers in a double-blind, randomized clinical trial evaluating the *in vivo* effect of hydrogen peroxide (35%) and peroxide carbamide (37%) six months after whitening.

Methods and Materials: Fifty volunteers participated, each with color change in a nonvital tooth. Fifty teeth were randomly divided into two groups (n=25), and the teeth were bleached using either 35% hydrogen peroxide (G1) or 37% carbamide peroxide (G2). Intracoronal bleaching was carried out by a technical "walking bleach" over four sessions. Gingival crevicular fluid samples were collected and used to quantify the IL-1β and RANK-L secreted levels. Samples of six periodontal sites (three vestibular and three palatal) were collected for up to six months (at the beginning of the study [baseline] and at one week, one month, and six months posttreatment). The color change was visually monitored using the Vita Bleached Guide (ΔSGU).

Results: Comparing each time to baseline assessment, a significant increase in the levels of IL-1β and RANK-L across time points was detected ($p<0.05$). The color change was 4 in G1 and G2, and a statistically significant difference ($p<0.05$) was found at the month time point between the groups. Using the Spearman test, a strong correlation (>0.8)

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between the IL-1β and RANK-L levels in both groups at all time points was detected.

Conclusions: Nonvital bleaching using a technical walking bleach induces an increase in the IL-1β and RANKL production in periodontal tissues, which persists for six months after treatment. Both biomarkers were highly correlated in both groups and at all time points.

INTRODUCTION

Whitening of nonvital teeth has proven to be an effective, minimally invasive treatment capable of restoring the esthetics of smile in cases that affect the self-esteem of the patient and his or her social interactions.[1,2]

High concentrations of hydrogen peroxide (H_2O_2; >30%), sodium perborate, and carbamide peroxide are commonly used for whitening. Each of these bleaching agents operates on the same oxidation mechanism: organic pigment by-products from the decomposition of H_2O_2.[3]

These whitening procedures have demonstrated significant success and patient satisfaction.[4,5] To the best of our knowledge, however, no follow-up of patients from a randomized clinical trial has been undertaken to analyze the possible adverse effects of whitening, such as external cervical resorption.[6] This effect is relatively unusual, but it typically leads to tooth loss.[7,8] The high oxidizing power of hydrogen peroxide can modify the histological and morphological properties of the tooth structure,[9] activate matrix metalloproteinases,[10] result in cytotoxicity, produce free radicals, and change the pH.[11] Similarly, a recent *in vitro* study demonstrated that bleaching results in an increase in the activity of matrix metalloproteinases and osteoclasts.[12,13]

Taken together, these data suggest that nonvital tooth bleaching could affect the levels of inflammatory markers, such as interleukin-1β (IL-1β) and the bone resorptive factor called receptor activator of nuclear factor kappa-B ligand (RANK-L), which have been associated with the regulation of resorption of the root.[14,15] A direct relationship between these two biomarkers during intracoronal bleaching has not been reported yet. Moreover, there have been no longitudinal follow-up studies about the effects of these biomarkers.

The purpose of this investigation was to assess the *in vivo* effect of hydrogen peroxide (35%) and carbamide peroxide (37%) on the levels of periodontal markers (IL-1β and RANK-L) up to six months postwhitening and to determine whether there is any correlation between their levels.

We hypothesized that the "walking bleach" technique increases the levels of IL-1β and RANK-L, which remains stable for up to six months after treatment. Also, we hypothesized that there is no correlation between these biomarkers.

METHODS AND MATERIALS

This clinical trial was randomized, double-blinded (patient and evaluation), and approved by the Ethics Committee of the Faculty of Dentistry of the Local University. The participants were informed of the goals and risks of the treatment as well as their freedom to leave the study at any time. Clinical procedures were explained in detail, and the subjects of the study were required to provide informed consent according to the regulations of the institutional Ethics Committee of the Faculty of Dentistry of the Local University. This research was conducted in full accordance with the World Medical Association Declaration of Helsinki of 1975 (revised 2000) and independently reviewed and approved by a local ethics committee/institutional review board.

Sample Size

We used the program 3.2 G-Power to determine the sample size (power statistic of 80%, 5% significance level, 15% abandonment). Participants were invited via social networks (Facebook and Twitter) to visit the dental school. Seventy-four volunteers were examined to assess whether they satisfied the study criteria. Fifty patients were selected for the study, and a total of 50 (1 per patient) discolored nonvital teeth satisfied the study criteria.

The inclusion criteria were patients aged at least 18 years with one or more nonvital teeth with color change of A2 or higher, without vestibular restoration covering the middle third of the tooth, no apical lesions, and no prior experience with whitening.

Exclusion criteria included pregnant or breastfeeding patients, the presence of enamel hypoplasia, spots of tetracycline or fluorosis, orthodontic braces, periodontal disease, or active caries lesions.

The volunteers were examined clinically and radiographically to determine the presence of periapical lesions, internal or external root resorption, dental caries, or periodontal disease. If any of these conditions was detected, the participants were briefed and referred to a specialist for treatment.

We divided the patients into two groups according to the bleaching agent used (n=25); the patients were divided randomly using Microsoft Excel (Microsoft, Seattle, WA, USA). Patients in group 1 (G1) were treated with 35% hydrogen peroxide (Opalescence Endo, Ultradent, South Jordan, UT, USA), and patients in group 2 (G2) were treated with 37% carbamide peroxide (Superendo Whiteness, FGM, Brazil).

Participants received a pumice and water prophylaxis. They also received oral hygiene instructions to maintain and enhance periodontal health status.

Bleaching Protocol

The bleaching agent was applied according to the manufacturer's instructions over four sessions via an outpatient technique; each session was separated by one week.

Preparation Session—The root canal was prepared under rubber dam isolation. 3-mm of endodontic filling was removed apical to the cementoenamel junction (CEJ), and a sealing of 2 mm was applied using a resin-reinforced glass ionomer cement (Riva Light Cure, SDI, Bayswater, VIC, Australia).

The cement was cured for 60 seconds at a distance of approximately 1 cm, with a 1200-mW lamp intensity (Cal Raddi, SDI). Due to the italic s-shape of dentin tubules in the cervical region, and to guarantee the whitening of this area of the tooth, the coronal limit of the seal was located 1 mm below the CEJ. The proper sealing of the endodontic treatment was verified radiographically.

A small amount of whitening gel remained in the pulp chamber in the presence of moisture (walking bleach technique). The cavity was sealed with temporary cement (Fermín, Detax, Germany) until the next session.

During the whitening sessions, the access cavity was washed with water and temporarily sealed for seven days before the final restoration with composite Brilliant (Coltene Whaledent AG, Altstätten, Switzerland).

Patients were instructed to avoid foods that can stain teeth, such as coffee, tea, or wine.

Evaluation of Color

Tooth color was visually recorded by two independent evaluators (with a Kappa test agreement of 80%). This procedure was done before (baseline) treatment and at one week, one month, and six months after the treatment had concluded. The evaluation of color was done in the middle third of the tooth according to the recommendations of the American Dental Association.[16] The patients were examined under the same conditions independently by each reviewer, using the color scale Vita Bleachedguide (Vita Zahnfabrik, Bad Säckingen, Germany). In case of disagreement, the two referees reached a consensus in the presence of the patient. Each color on the scale was assigned a numeric value from which we could compute scalar color changes in units (ΔSGU).

Quantification of IL-1β and RANK-L Levels in Gingival Crevicular Fluid

After the tooth was isolated with a cotton roll, the supragingival plaque was removed using a Gracey 3/4 curette without touching the marginal gingiva. The gingiva was dried gently using air from a syringe, and the gingival crevicular fluid (GCF) was collected using paper strips (Periopaper, Ora-Flow Inc, New York, NY, USA) placed in the periodontal sulcus, and left in position for 30 seconds. Paper strips contaminated with saliva or blood were discarded. The GCF samples of six periodontal sites were obtained: three vestibular and three palatal (mesial, middle and distal), before the whitening treatment (baseline) and at one week, one month, and six months after the whitening treatment. The GCF samples were then eluted twice in 120 μL of buffer saline with 0.05% Tween-20 (Fluka, Sigma Aldrich Chemie GmbH, Buchs, Switzerland), centrifuged at 10,000 g for five minutes at 4°C, and stored at −80°C until analysis.

Total protein levels were quantified using the Bradford method (R&D Systems Inc, Minneapolis, MN, USA), and 100 μL elution was used for RANK-L and IL-1β quantification using an enzyme-linked immunosorbent assay–based assay (Quantikine test, R&D Systems). Absorbance was measured at 450 nm with a correction at 540 nm for RANKL and 620 nm for IL-1β, using an automated plate reader (ELx800, Bio-Tek Instruments Inc, Winooski, VT, USA). The concentration of each marker in each sample was calculated using a four-parameter logistic equation.

Statistical Analysis

Statistical analyses were performed using SPSS version 25.0 (SPSS Inc, Chicago, IL, USA) with an α-value of 0.05 indicating statistical significance. For intragroup analysis, we used the Wilcoxon test; the Mann-Whitney test was used for analyzing the variables between the groups. Correlations were calculated using Spearman R in Graph Pad Prism

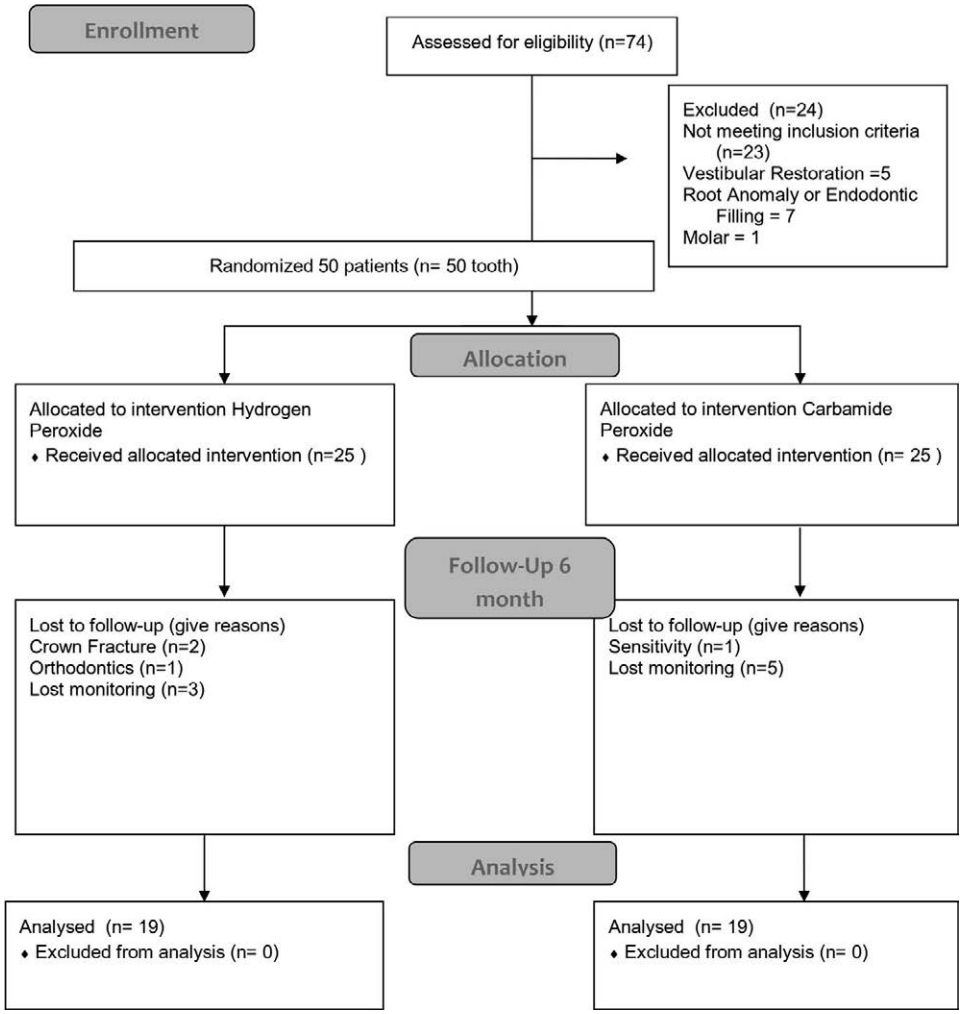

Figure 1. *Flow diagram of the clinical trial, including detailed information on the excluded participants.*

version 7.00 (Graphpad , La Jolla, California, United States).

RESULTS

Six months after treatment, 38 of the 50 recruited patients completed treatment and satisfied the study criteria, resulting in a final sample of 38 teeth (19 teeth per group; Figure 1). The characteristics of the final sample are reported in Table 1.

Subjective Color Measurement

A statistically significant difference in color ($p<0.05$) was detected at the one-month time point. The teeth in the G1 group were bleached a bit more than those in the G2 group (Table 2).

Levels of IL-1β and RANKL

The biomarker data were analyzed using a media by tooth collection or pooled complete data; the statistical results were similar, so we decided to present the data as a pool. Six sites per tooth were measured (114 sites for the hydrogen peroxide group), and 113 sites were measured in the carbamide peroxide group. Table 3 lists the levels of IL-1β (pg/μL). All of the time points exhibited a significant difference ($p<0.05$). Table 4 lists the levels of RANK-L

Table 1: *Baseline Features of the Participants*

Baseline Feature	Group	
	Hydrogen Peroxide	Carbamide Peroxide
Age, y (mean ± SD)	30.6 ± 11.7	30.8 ± 11.3
Minimum age, y	19	20
Maximum age, y	65	65
Male, %	47.83	39.13
Trauma, %	56.52	39.13
Caries, %	43.48	60.87
VITA Bleachedguide 3D-MASTER Median (minimum;maximum)	12 (7;15)	11 (9;15)

Table 2: *Comparison of ΔSGU Values at Different Time Points Using the Vita Bleach Guide (Mean ± SD)*

Assessment Point	Color Change by ΔSGU		
	Hydrogen Peroxide	Carbamide Peroxide	Mann-Whitney Test
Baseline vs one week after bleaching (before restoration)	5.00 ± 2.26[a]	4.16 ± 1.26	0.093
Baseline vs one week after bleaching (after restoration)	5.00 ± 2.24[a]	4.16 ± 1.26	0.080
Baseline vs one month after bleaching	4.84 ± 2.14[a]	3.89 ± 1.20	**0.037**
Baseline vs six months after bleaching	4.37 ± 2.29	3.89 ± 1.15	0.180

[a] *Statistically significant intragroup difference (Wilcoxon test, p<0.05), baseline vs six months after bleaching.*

expressed in pg/μL for all of the sites. According to the Wilcoxon test, the measurements of all time points were significantly different from those obtained at baseline ($p<0.05$). Using the Mann Whitney test, we did not detect any significant difference between the two groups ($p>0.05$). Figure 2 shows the linear correlation between the two biomarker tests (Spearman test); all correlations were greater than 0.8 with a p-value less than 0.0001 (all time points) and for both treatment groups (Figure 2).

DISCUSSION

Intracoronal bleaching is a very efficient and minimally invasive protocol that is currently widely used by endodontists to solve esthetic problems and achieve high levels of patient satisfaction.[17] Our results also demonstrate high effectiveness in the average of the color change units (ΔSGU; Vita Bleached Guide). However, there are safety concerns associated with this process (eg, hydrogen peroxide has been reported to be cytotoxic).[18,19] Another study reported a resorptive increase in the proliferation and activity of osteoclasts after bleaching.[13] This study is the first randomized clinical study to assess the biological effects of intracoronal whitening. Our data show that intracoronal bleaching results in an increase in the levels of RANKL and IL-1β that persists for six months after whitening.

RANKL is part of the RANK/RANKL/OPG axis that regulates bone metabolism.[20] We also detected an increase in the levels of IL-1β for six months after whitening. The levels of IL-1β and RANK-L were highly correlated among them and during all the periods assessed. We detected a positive and proportional correlation of both markers that increased during the sessions and persisted for up to six months of follow-up. Both biomarkers are recognized to be very sensitive to inflammation and the resorption process. Our results are conclusive, and the first hypothesis is accepted whereas the second was rejected, because the levels of markers increased and persisted up to six months after treatment. In addition, there was a positive correlation between them.

Even though this study cannot answer whether root resorption is produced by intracoronal bleaching, it provides some clues that could help clarify the question.

Comparing treatments, hydrogen peroxide was more effective (color change) than carbamide peroxide at all time points up to six months after whitening (Mann-Whitney test, $p<0.05$; Table 2). There was a little rebound at the sixth month, consistent with the results of previous studies of bleaching in nonvital teeth.[21,22] The greater efficacy of hydrogen peroxide may be due to its greater availability; carbamide peroxide should first be

Table 3: *IL-1β Levels Expressed (pg/μL), Mean ± SD[a]*

Assessment Point	Hydrogen Peroxide	Carbamide Peroxide	Δ Hydrogen Peroxide	Δ Carbamide Peroxide	Mann-Whitney Test
Baseline	102.20 ± 48.61	114.81 ± 52.50			
One week after bleaching	160.02 ± 70.74[b,c]	174.91 ± 76.71[b,c]	60.06 ± 31.09	60.11 ± 42.02	0.323
One month after bleaching	177.35 ± 76.00[b,c]	193.74 ± 83.26[b,c]	77.10 ± 36.27[c]	78.93 ± 47.79[c]	0.709
Six months after bleaching	192.19 ± 79.17[b,c]	213.20 ± 88.79[b,c]	91.97 ± 39.19[c]	98.39 ± 52.42[c]	0.581

[a] *Δ indicates difference between time point and baseline value.*
[b] *Statistically significant difference from baseline using the Wilcoxon test (p<0.05).*
[c] *Statistically significant difference from the previous time using the Wilcoxon test (p<0.05).*

Table 4: *RANKL Levels Expressed (pg/μL), Mean ± SD*[a]

Assessment Point	Hydrogen Peroxide	Carbamide Peroxide	Δ Hydrogen Peroxide	Δ Carbamide Peroxide	Mann-Whitney Test
Baseline	13.03 ± 5.88	14.04 ± 5.77			
One week after bleaching	25.70 ± 11.46[b,c]	25.89 ± 10.42[b,c]	13.68 ± 7.28	11.84 ± 5.88	0.078
One month after bleaching	28.67 ± 12.40[b,c]	29.32 ± 11.36[b,c]	16.70 ± 8.25[c]	15.28 ± 6.87[c]	0.201
Six months after bleaching	31.77 ± 12.85[b,c]	33.44 ± 12.55[b,c]	19.75 ± 9.02[c]	19.40 ± 8.28[c]	0.755

[a] *Δ indicates difference between time point and baseline value. Δ (assessment points vs baseline).*
[b] *Statistically significant difference from baseline using the Wilcoxon test ($p<0.05$).*
[c] *Statistically significant difference from the previous time using the Wilcoxon test ($p<0.05$).*

decomposed (10% carbamide peroxide yields approximately 3.5% of hydrogen peroxide), resulting in a lower concentration of hydrogen peroxide, the active ingredient of whitening.

Biomarkers did not attain the levels of active periodontal disease.[23] However, the detected levels may be sufficient to generate some alteration in the most susceptible individuals. It is very striking that the markers were present six months after treatment. This finding implies that the stimulus generates a mediating effect, which has not been reported in the literature.

Patients with a history of periodontal disease may be more susceptible to the imbalance of these markers, which would generate a contraindication for this type of treatment. To the best of our knowledge, this has not been described in the literature.

IL-1β is an initiator of potent activity of the osteoclasts[24] and stimulates macrophages and endothelial monocytes (among others) to produce matrix metalloproteinases, prostaglandins, and other proinflammatory cytokines.[24,25] IL-1β also stimulates osteoblasts to produce RANKL, causing differentiation and maintenance of osteoclasts.[23]

External cervical resorption is another important relevant aspect in nonvital teeth,[1] which, despite its low incidence, has been described as a possible adverse effect of intracoronal whitening, and almost always leads to tooth loss. The cause is not clear, and the results of this work can offer a clue as to how the process of resorption is activated from the RANK/RANKL/OPG axis, which also regulates the process of resorption of the root.[20,23] It should be noted that there is significant anatomic variability between endodontically treated teeth; anatomical defects can lead to wider dissemination of the peroxide in the space of the root, creating an inflammatory process that could become chronic and culminate in a resorptive process. Whitening treatments have been lessening in their concentrations and are free of

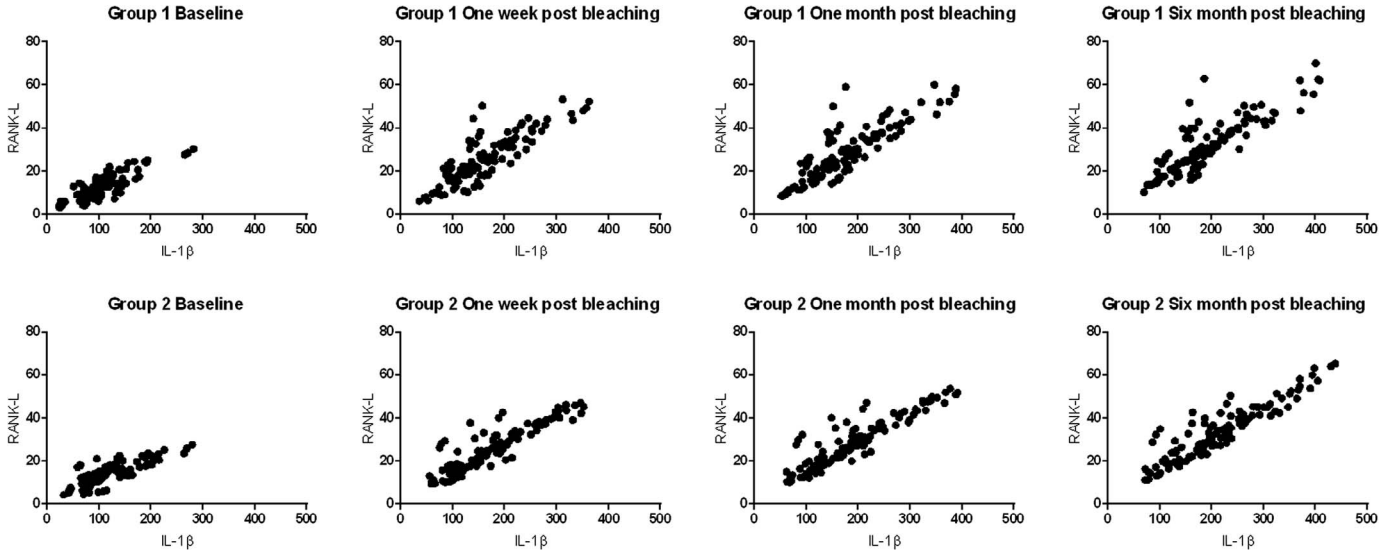

Figure 2. *Linear correlation between two biomarkers (Spearman test).*

activators such as heat, which is used to accelerate chemical reactions and obtain faster results.

The technique of walking bleach uses a closed chamber,[26] wherein the chemical agent is in contact with the tooth for a prolonged length of time. A closed-chamber pressure favors the dissemination of peroxide into the extraradicular space.

A limitation of this study was its inability to have real negative controls. Unfortunately, that protocol was not approved by the local ethics committee in any form (instead of a placebo within the tooth, serum, or something similar). However, comparisons with the reference data were significant for both biomarkers at all time points. On the other hand, the strong correlation between biomarkers suggests a negative effect of the whitening gels that persisted for weeks after the treatment. Despite not being the objective of this work, the question remains whether the cement used to seal the canal could be a factor in the persistence of inflammation.

CONCLUSIONS

Nonvital bleaching with hydrogen peroxide (35%) or carbamide peroxide (37%) generates an imbalance in the levels of fluid gingival RANK-L and IL-1β persisting up to six months after treatment with a strong and positive correlation between the biomarkers. Hydrogen peroxide is more effective than carbamide peroxide bleaching, but the color change was stable in both groups for up to six months.

Acknowledgements

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Regulatory Statement

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

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Detection of Approximal Caries Lesions in Adults: A Cross-sectional Study

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M Lira • J Sánchez • G Rodríguez • S Osorio • ME Ortiz

Clinical Relevance

Direct visual inspection following temporary tooth separation of approximal lesions that reach the D1-D2 of dentin may contribute to a better decision making about restorative treatment.

SUMMARY

Detection and management of posterior approximal caries lesions are still problematic. Inspection of approximal surfaces is challenging, and bitewing radiographs are used when direct vision is not possible. Unfortunately, there is no definite radiographic appearance to identify lesion cavitation with absolute certainty. Many lesions detected radiographically within the outer half of dentin are not cavitated, often resulting in unnecessary restorative treatment. Our study compared radiographic depth of approximal caries lesions with presence of cavitation in adults using visual inspection following temporary tooth separation (TTS). We conducted this observational descriptive cross-sectional study at two dental schools in two cities in Chile. Clinicians were unaware of radiographic depths of lesions and examined 147 participants (57.3% female and 42.7% male) following TTS. Using the common classification system that consists of E0 (no lesion), E1 (lesion within the outer half of enamel), E2 (lesion within the inner half of enamel), D1 (lesion within the outer third of dentin), D2 (lesion within the middle third of

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dentin), and D3 (lesion within the inner third of dentin), a trained dentist evaluated all the processed films. Cavitation was detected in only three sites (0.22%) within the E0 category, seven sites (3.41%) in E1, five sites (14.8%) in E2, four sites (14.8%) in D1, six sites (50%) in D2, and eight sites (61.5%) in D3. Considering that restorative treatment should be indicated strictly for cavitated lesions, our findings support indication for restorative treatment for D3 lesions and the rationale for TTS for D1-D2 caries lesions to allow direct visual inspection to determine whether there is surface cavitation.

INTRODUCTION

The detection and management of approximal caries lesions in areas of posterior tooth contact are still problematic for clinicians. Direct vision of approximal surfaces is challenging; hence, radiographic examination of these surfaces is fundamental.[1] Bitewing radiography is used to evaluate approximal lesions that cannot be readily identified clinically. This also allows estimating lesion depth and its relationship with the dental pulp and permits monitoring lesion progression. These are essential aspects in the decision-making process when considering treatment.[2]

Unfortunately, there is no definite radiographic appearance to identify the presence of cavitated lesions with absolute certainty.[1,3] Nevertheless, bitewing radiographs are still widely used and present a far better alternative when compared with clinical examination without radiographic examination.[4] There is great variation across studies between radiographic images and the presence of cavitation. Many lesions detected radiographically within the outer half of dentin are not cavitated.[5-7] Also, there is a great deal of variation from one clinician to another in the decision-making process regarding treatment of approximal caries lesions, which may often result in invasive treatment at an early stage.[8] Restorative treatment of approximal surfaces leads to the removal of large areas of sound enamel, causing structural damage to the tooth through loss of marginal ridges and initiating a re-restorative cycle for the tooth. There is also a potential risk of further damage to adjacent surfaces.[9,10] With all this in mind, it is relevant to determine the relationship between the clinical status of caries lesions, their level of radiographic radiolucency, and their correlation in terms of cavitation. This would improve the indications for noninvasive as well as restorative treatment for approximal caries lesions and allow making the right therapeutic recommendations.

The aim of this study was to compare the radiographic depth of approximal caries lesions with the presence of cavitation by means of visual inspection following temporary tooth separation (TTS) in adult patients.

METHODS AND MATERIALS

This observational descriptive cross-sectional *in vivo* study was performed as a diagnostic test on previously consented patients at the dental clinics of the Faculty of Dentistry (University of Chile, Santiago, Chile) and the Dental Institute (Austral University, Valdivia, Chile). Both institutions openly provide dental care within the community as part of their academic activities.

All participants were informed and gave their consent to take part in this study in accordance with the Declaration of Helsinki. Participants signed an informed consent form previously approved by the relevant ethics committees.

The following surfaces of permanent teeth were included in this study:

- Mesial surfaces of second premolars, first molars, and second molars
- Distal surfaces of first premolars, second premolars, and first molars

The following surfaces were excluded:

- Approximal surfaces with no approximal contact area or without an adjacent tooth
- Restored approximal surfaces or those adjacent to restored approximal surfaces
- Dental or maxillofacial anomalies precluding examination of interproximal spaces

A trained operator took conventional bitewing radiographs as part of the radiographic examination using double films (D57, Ultraspeed, fine grain, Kodak, Rochester, USA). The exposure of all films was standardized. Intraoral radiograph holding devices were used to keep intraoral films in place and help position the X-ray tube. These devices allow standardizing the angulation of the X-ray beam and facilitate taking reproducible radiographic views. The exposure time and settings were the same for both premolar and molar teeth: 0.4 seconds, 70 kV, and 8 mA. The same operator who took the radiographs processed the films using an automatic

developer (Periomat Plus 1307/1308, Dürr, Bietigheim-Bissingen, Germany) according to the manufacturer's instructions. Using the common classification system that consists of E0 (no lesion), E1 (lesion within the outer half of enamel), E2 (lesion within the inner half of enamel), D1 (lesion within the outer third of dentin), D2 (lesion within the middle third of dentin), and D3 (lesion within the inner third of dentin), a trained dentist evaluated all the processed films.[11] Radiographs were assessed using a light box under optimal conditions.

A dental radiologist collated and recorded radiographic data in a *pro forma* form, subsequently highlighting the proximal surfaces of teeth that conformed to the inclusion criteria of this study, recording in writing the corresponding code (E0, E1, E2, D1, D2, D3) for each approximal surface. Clinical operators were unaware of the radiographic depths of caries lesions. Coded data from these forms were stored in a computer file (Excel for Windows) and later analyzed using data analysis software (Stata 11.0, Stata Corp, Texas, USA). A descriptive analysis of data was performed. The percentage of sites according to proximal caries lesion depth on bitewing radiographs was calculated.

Participants underwent TTS with orthodontic elastic bands (AlastiK radiopaque separators, 3M Unittek, St. Paul, Minnesota ,USA) in premolars and molars for three days[12] according to the inclusion criteria. Following TTS, interproximal spaces were cleaned after the removal of the separator using water spray and dental floss (Oral-B, Santiago, Chile). Teeth were clinically examined immediately after cleaning using direct vision in order to check for the presence or absence of surface cavitation. Tactile examination was also performed using the World Health Organization probe developed for the *Community Periodontal Index of Treatment Need* method. Suitably trained clinicians who had been previously calibrated in the detection of dental surface cavitation visually examined all temporarily separated approximal surfaces. For the purpose of this study, presence of cavitation was established as a break in continuity in enamel detectable on visual examination. Examinations were performed using dental chairs with relative isolation (cotton swabs and saliva ejector), using dental mirrors, three-in-one syringes to dry the teeth, and artificial light. No dental loupes were used. The presence and proportion of approximal caries lesions following visual inspection and their relationship with radiographic depth were calculated. The percentage of sites with cavitated approximal lesions according to visual inspection was also calculated.

RESULTS

The number of approximal surfaces included in this study according to the inclusion criteria was 1937. Two hundred and twenty approximal sites (11.4%) were excluded from the study: 15.7% in Santiago and 7.2% in Valdivia. Reasons for exclusion from the study included patients who abandoned dental treatment or those for whom full data were not obtained, for example, due to lack of compliance, loss or self-removal of the orthodontic elastics, or presence of sites where clinical examination proved inaccessible. Thus, the total number of approximal surfaces examined clinically was 1717.

The number of participants was 146 with an average of 11.7 (SD 6.7) approximal surfaces evaluated and an average age of 25.2 (SD 8.8) years; 57.3% of participants were female, and 42.7% were male. Within our sample, 75.2% came from the dental clinic at Austral University and 24.8% from the dental clinic at the University of Chile.

Depth of Approximal Caries Lesions Detected on Bitewing Radiographs

Of the total number of approximal surfaces evaluated by means of bitewing radiography, 70.8% corresponded to healthy sites (E0), whereas 19.7% displayed radiographic evidence of caries and lesion presence of varying depths. Visual inspection on clinical examination of approximal surfaces post-TTS revealed 33 sites with cavitated caries lesions. When relating the presence of identified cavitated approximal caries lesions according to visual inspection to recorded radiographic depths, cavitation was detected in only three sites (0.22%) within the E0 category, seven sites (3.41%) in E1, five sites (14.8%) in E2, four sites (14.8%) in D1, six sites (50%) in D2, and eight sites (61.5%) in D3 (see Table 1). We observed a high proportion of lesions that were radiographically into dentin (D1-D3) and that were deemed noncavitated caries lesions; 34 of 52 (65.4%) lesions identified as affecting dentin by radiograph were noncavitated lesions.

DISCUSSION

Detection of dental caries using bitewing radiographs provides information about the presence and depth of approximal lesions. However, the use of radiography presents limited sensitivity in the detection of cavitation.[13,14] Hence, the use of elastic

Table 1: *Number of Approximal Cavitated and Noncavitated Caries Lesions on Direct Visual Inspection and Their Distribution According to Different Categories of Radiographic Depth*

Direct Visual Inspection	Categories of Caries Lesion Severity on Radiographic Diagnosis (n [%])						
	E0	E1	E2	D1	D2	D3	Total
Noncavitated	1375 (99.8%)	198 (96.6%)	77 (93.9%)	23 (85.2%)	6 (50%)	5 (38.5%)	1684 (98.1%)
Cavitated	3 (0.2%)	7 (3.4%)	5 (6.1%)	4 (14.8%)	6 (50%)	8 (61.5%)	33 (1.9%)
Total	1378	205	82	27	12	13	1717

Abbreviations: E0, no lesion; E1, lesion within the outer half of enamel; E2, lesion within the inner half of enamel; D1, lesion within the outer third of dentin; D2, lesion within the middle third of dentin; D3, lesion within the inner third of dentin.

bands has been proposed in order to achieve interproximal separation and allow visual inspection of interproximal sites. This reversible and low-cost method eliminates interproximal contact points between adjacent teeth and thus improves the performance of conventional visual inspection.[15,16] The aim of this *in vivo* study was to compare the radiographic depths of approximal caries lesions with the presence of cavitation by means of direct visual inspection of surfaces in molar and premolar permanent teeth in adults following a period of TTS to determine the relationship between the stage of clinical progression of caries lesions and the level of radiolucency observed in the radiographs taken.

Our sample of 1717 surfaces was representative of the Chilean adult population in terms of gender and came from two cities engaged in tap water artificial fluoridation programs (Santiago: 0.62 ppm fluoride; Valdivia: 0.939 ppm fluoride).[17,18] Reasons for exclusion of approximal surfaces from the study included incomplete recording of data in situations where inadequate tooth separation was attained due to participant withdrawal from the study or loss of elastics, resulting in an 88.6% of retention rate of surfaces examined. In order to address this issue, 10% oversampling was built into the study design. There are no reasons to think either that this loss rate could have resulted in a source of bias, which in turn could affect the results, or that there were different characteristics between approximal sites that were lost and those that remained in the study. As the radiographic depth of caries lesions increased, we observed an increase in the proportion of approximal lesion cavitation.

The majority of approximal caries lesions were not cavitated. Preventive treatment is therefore indicated in these cases. The proportion of approximal cavitated lesions detected by visual inspection after TTS is directly related to the radiographic depth. As radiographic depth increased, so did the number of cavitated sites (Table 1), which concurs with Nielsen and others;[13] who emphasized the relationship between caries lesion depth and the probability of cavitation presence. It follows that the probability of cavitation within an approximal enamel lesion is low, and it would be incorrect to assume all radiolucent lesions in dentin to be cavitated.[3,19] According to our results, there was a small number of cavitated sites within the E0 category. This might have been due to limitations inherent to the radiographic technique used in the detection of interproximal caries lesions in our study.

Since the 1970s, several clinical studies have been developed with the aim to relate radiographic appearance of caries lesions to the probability of cavitation presence. Methodologies used in other studies differ from the one used in our study. Some authors evaluated approximal surfaces using study casts following TTS and impression making.[20-22] Other studies looked at approximal surfaces at the time of tooth extraction.[23] Others evaluated approximal surfaces at the cavity preparation stage, which raises the question of whether operator error may have resulted in unintended rotary instrument contact against approximal surfaces causing accidental cavitation, thus altering their results.[8,19,24]

When comparing our study with other clinical studies of similar methodology, it is possible to see our results agree with those of Pitts and Rimmer[3] and Hintze and others[6] given that similar percentages of cavitated surfaces were found radiographically in categories R0, R1, R2, R3, and R4.[5,25,26] We also looked at percentages of cavitated surfaces in relation to radiographic depths comparable with the studies mentioned previously. Our study determined a lower proportion of cavitated surfaces in stages D1 and D2 compared with those earlier reported by others. The percentage of cavitated surfaces detected in this study proved lower than those reported by other studies.[5,23,25,26] Reasons for these differences might include the relatively younger age-group within our study and the exposure of participants to fluoridated tap water. With regard to the work of Coutinho and daRocha

Costa, it was not possible to draw comparisons because their study used different categories of radiographic depth and also evaluated approximal lesions of deciduous molars.[27] It is worth highlighting that except for histological sectioning, which is considered to be the true gold standard for detecting demineralization of dental tissues, no other available method for caries detection is able to attain such results.[3]

Our results are clinically relevant, as they show that the probability for an approximal enamel lesion to be cavitated (E1 or E2) is very low. Hence, the management of these lesions should be based on plaque control, application of remineralization therapies, and timely clinical as well as radiographic monitoring. Results also confirm once again the importance of preventive and noninvasive treatment. Most approximal caries lesions in posterior permanent teeth were not cavitated and therefore do not require restorative treatment, especially at the D1 level. Our results support current evidence-based clinical recommendations to restore lesions only when cavitation is present. Postponing operative intervention avoids committing teeth to an endless restorative cycle and generates biological benefits for patients, enabling them to keep their teeth for longer throughout their lives.[28] Historically, the use of dental services in public health has not created great benefits in terms of a decrease in caries incidence. A small contribution (3%) of dental services to the decline of the disease has been reported, while a 65% decrease has been associated with socioeconomic factors and access to fluoride.[29] The role of dentistry in decreasing the prevalence and incidence of dental caries should focus on early lesion prevention and remineralization in order to delay any operative intervention for as long as possible. Approximal restorative treatment should be performed only in the presence of cavitation, as in those circumstances biofilm removal within the cavity would not be possible by otherwise conventional means (brushing and flossing or other interproximal cleaning), thus posing a higher risk of lesion progression. TTS is an adjunct to clinical and radiographic detection of caries lesions, particularly when lesions are confined to dentin at the D2 and D3 levels given the probability of cavitation and therefore the need for restorative treatment. Hence, using TTS in lesions confined to dentin (D2-D3) should assist clinicians in determining the presence of surface cavitation and helping to establish the optimal timing for restorative treatment when required.

As far as we know, this is the first study in adults in Latin America that correlates the presence of approximal caries lesions detected visually and radiographically to the presence of cavitation in adult patients. Our study builds on prior research in this field.

CONCLUSIONS

In this study, only 14.8% of lesions in category D1 were cavitated on clinical inspection, and in category D2 the proportion was 50%, while in D3 the proportion was 61.5%. Taking into consideration that restorative treatment should be indicated strictly for cavitated lesions, our clinical recommendations based on our results are the following:

- D3 radiographic caries lesions are more likely to be cavitated; therefore, restorative treatment is indicated.
- It is highly recommended that D1 and D2 radiographic caries lesions undergo TTS in order to increase diagnostic accuracy and decide whether to perform restorative treatment.

Acknowledgements

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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Influence of Staining Solutions on Color Change and Enamel Surface Properties During At-home and In-office Dental Bleaching: An *In Situ* Study

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Clinical Relevance

The consumption of cola and coffee can affect the color change of enamel during both at-home and in-office bleaching treatment. In addition, the consumption of cola changes the microhardness, roughness, and micromorphology of the enamel surface.

SUMMARY

The purpose of this *in situ* study was to evaluate the influence of staining solutions (coffee and cola) on the color change, microhardness, roughness, and micromorphology of the enamel surface during at-home and in-office dental bleaching. One hundred and thirty-five enamel bovine blocks were prepared to perform the evaluations. Fifteen volunteers used an intraoral appliance with nine enamel blocks for 15 days. The enamel blocks were randomly assigned among the different groups according to the three treatments: in-office bleaching with high hydrogen peroxide concentration (Opalescence Boost PF 40%, Ultradent) for 40 minutes in three sessions (first, eighth, and 15th days of treatment), at-home bleaching with low carbamide peroxide concentration (Opalescence PF 10%, Ultradent) for 60 minutes daily for 15 days, and a control group (no bleaching agent applied). The enamel blocks were immersed daily in different staining solutions (coffee or cola) for 30 minutes for 15 days or were not submitted to staining (control) to obtain a factorial scheme

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(3×3) of the dental bleaching treatment and staining solution (n=15). The microhardness analyses (Knoop), roughness evaluations (Ra), surface micromorphological observations, and color measurements (using the CIELAB system and the VITA Classical scale) were made before and after the bleaching treatments to assess immersion in staining solutions. Mixed model tests showed that there was a decrease in enamel microhardness after exposure to cola compared with coffee and the control group ($p<0.0001$) for both bleaching techniques. Roughness was higher for the cola groups ($p<0.0001$), and there was no significant difference between the coffee and the control groups. Generalized linear models showed that when no staining solution was applied, lighter color scores were found for the VITA Classical scale ($p<0.0001$). Without the staining solutions, there was an increase in luminosity (ΔL) ($p=0.0444$) for in-office bleaching. Lower values of Δa ($p=0.0010$) were observed when the staining solutions were not used. The Δb ($p=0.3929$) did not vary significantly between the bleaching agents, but when cola was applied, the values were significantly higher than for the control ($p=0.0293$). Higher values of ΔE ($p=0.0089$) were observed for in-office bleaching without staining solutions, while lower values of ΔE were observed for the in-office associated with coffee immersion. Regardless of whether being submitted to bleaching, the enamel stained with cola showed a decrease in microhardness, an increase in roughness, and changes in the micromorphology. The efficacy of the bleaching agents was greater when no staining solution (cola or coffee) was used, and in-office bleaching showed greater color change than the at-home bleaching technique.

INTRODUCTION

The color of dental elements can be modified noninvasively with dental bleaching procedures using either carbamide peroxide or low-concentration hydrogen peroxide (in the at-home treatment) or else using high concentrations of hydrogen peroxides (for in-office bleaching), which leach out the chromophores present in the dental structure.[1] In dental bleaching, carbamide or hydrogen peroxides release oxygen and perhydroxyl free radicals, capable of diffusing through the dental structure, breaking down the large and pigmented molecules (chromophores) into smaller molecules, modifying the reflection of the wavelength of light, and consequently altering tooth coloration.[2]

In addition to color change, changes of a micromorphological nature may be associated with the use of bleaching agents of low or high concentration, including enamel erosion or porosity[3-6] and greater enamel surface roughness[3,6-8] and permeability.[9] These changes may be associated with decreased enamel microhardness and loss of mineral content.[4,8,10] They occur due to the composition of bleaching agents, concentration of the peroxide used, pH, application time, storage medium (water or saliva), and protocols of the bleaching techniques. In this respect, surface micromorphology changes have been observed when using agents with higher concentrations of hydrogen peroxide[9] and lower pH values.[5,11] These surface changes could lead to greater roughness and consequent higher adhesion of biofilm and pigments to the enamel,[12] thus compromising bleaching efficacy.

For this reason, patients undergoing dental bleaching have been cautioned to avoid food and drinks rich in coloring agents, such as coffee, red sauces, red wines, tea, and chocolate.[13] Although Matis and others[14] observed that the consumption of pigmented food and beverages was not related to less bleaching effectiveness, they did not evaluate a control group with patients who did not have a diet free of pigmented foods or beverages for comparative purposes. An *in vitro* study by Liporoni and others[15] found that the consumption of wine after tooth whitening with 35% hydrogen peroxide led to the staining of the enamel structure. In contrast, Attin and others[16] demonstrated that a tea solution used for 10 minutes at different time intervals during bleaching with 10% carbamide peroxide did not influence the enamel color.

Among the pigmented drinks consumed by the population, coffee has high penetration and presence in Brazilian and American households. In 2017, 1.07 tons were consumed, putting Brazil right behind the United States in terms of consumption.[17] Cola-based soft drinks, especially Coca-Cola, also have broad population penetration—worthy of note, it is one of the most well known brands in the United States and ranked first among the best beverages in 2010.[18]

Coffee and cola are listed as the leading solutions causing tooth staining.[19-22] This is because both solutions present pigments that are adsorbed or absorbed by the substrate[23] in addition to having low pH values that may potentiate dental staining: coffee

is described as having a pH of about 5.5 [24] and cola of about 2.6.[21]

Some *in vitro* studies have evaluated the staining caused by coffee or cola associated with the bleaching treatment. When performing the bleaching technique with trays, Attia and others[19] observed lower color stability of the dental element when it was exposed to coffee during tooth whitening with 16% carbamide peroxide for 28 days. Coffee also caused staining of the enamel after dental bleaching with 6% hydrogen peroxide in the study by Bazzi and others[20] De Araújo and others[21] observed enamel that was stained with cola for one hour after bleaching with 10% hydrogen peroxide for 21 days. In regard to the in-office bleaching technique, only Mori and others[22] evaluated *in situ* the effect of coffee staining after bleaching with 35% hydrogen peroxide and found no influence on color change. Briso and others[25] also evaluated *in situ* the effect of staining with coffee and grape juice during bleaching with 10% carbamide peroxide and observed no difference in the final color at the end of the treatments. In a clinical study, Rezende and others[13] showed that exposure to coffee during bleaching treatment does not seem to affect the degree of at-home bleaching. However, no *in situ* study has evaluated the effect of coffee and cola on at-home and in-office dental bleaching treatments. In an *in situ* study, which is considered a preclinical study, the constant presence of saliva may influence the surface characteristics of dental enamel bleaching and have a remineralizing effect, and saliva may also deposit pigments on the dental surface. The null hypotheses to be tested were that there are no differences in enamel's 1) microhardness, 2) roughness, 3) micromorphology, or 4) color change between at-home and in-office dental bleaching submitted to the tested staining solutions (coffee and cola) or not submitted to any solution.

METHODS AND MATERIALS

Sample-Size Calculation and Patient Selection

Fifteen participants of both sexes were selected for this *in situ* study. The participants were selected after performing a sample-size calculation, considering the experimental design as one of repeated measures, in a 3 × 3 factorial scheme (solutions and bleaching agents). The inclusion of 15 participants enabled a high degree of freedom (269) and error (112) for the factors under study of "bleaching agent," "solutions," and the interaction of "bleaching agents vs solutions." The error was also high (126) for the "time" factor and for the interactions among "time" and the other factors. The G*Power software[26] for this experimental design (n=15) indicated an effect size higher than 0.80 for a 5% level of significance and a mean effect size of 0.25.[27]

Participants were included or excluded from the study based on history taking and clinical examination. The inclusion criteria were between 18 and 30 years of age and the presence of at least 24 sound teeth. The exclusion criteria were[28] people wearing dentures or fixed/removable orthodontic appliances, pregnant or breast-feeding women, smokers, and patients using medication that could reduce their salivary flow.

Four men and 11 women having a mean age of 21.7 years participated in this study. An impression of the upper arch was made for each volunteer, and a mold was obtained in dental stone on which a removable acrylic intrabuccal device for the palate was placed. It contained niches of a little over 5 × 5 × 3 mm to position nine blocks of enamel.

Preparation of Enamel Slabs

Sixty freshly extracted bovine incisors were obtained, cleaned, and stored in the freezer at -18°C until the experimental phase but no longer than two months prior. Before use, the teeth were thawed, and the roots were removed from the coronary portion with double-faced diamond discs (KG Sorensen, Barueri, Brazil). The crowns were sectioned to obtain dental blocks (5.0×5.0×3.0 mm) that were embedded in polyether resin (Maxi Rubber, Campinas, Brazil) and polished (Ecomet 250 grinder, Buehler Ltd, Lake Bluff, IL, USA) with adhesive abrasive paper (Buehler) using a granulation of 600 and then 1200. The polishing was performed with alumina suspension at 0.3 μm (Alfa Micropolish, Buehler) and a felt cloth (Buehler). The enamel blocks were cleaned in an ultrasound device with deionized water for 10 minutes to remove any residues as a result of the polishing procedure. Afterward, the enamel blocks were sterilized with ethylene oxide.[25] Two hundred enamel blocks were obtained at the end of this phase.

Microhardness Measurements

Three Knoop microhardness indents were made before applying the treatments, 150 μm from each other, on the top surface of each enamel slab using a microhardness tester (HVS-1000, Panambra, São Paulo, Brazil) under a 25*g* load applied for five seconds. The blocks selected presented microhard-

ness values closest to the mean (376.44±41.89 kgf/mm^2).

Roughness Measurements

Initial roughness measurements were performed with a profilometer (Surftest SJ-210, Mitutoyo, Suzano, Brazil) on the surface of the dental slabs. The mean roughness (Ra) was measured with a static load of 5 N and speed of 0.05 mm/s. The cutoff value was 0.25 μm in a sequential mode, and the measurement distance was 2.5 mm. Three tracings were made on each specimen at different locations, and the arithmetical mean was calculated. The values were obtained and tabulated. The mean value was obtained by selecting blocks of enamel with roughness values closest to the mean (0.04±0.01 μm). At the end of this phase, 138 enamel blocks were selected for the experiment (135 for the microhardness, roughness, and color analysis and three for the baseline micromorphological analysis).

Color Analysis

The initial color of the enamel slabs was evaluated with a spectrophotometer (Easyshade Advance, VITA, Bad Säckingen, Germany) whose tip (with a 5.0-mm diameter) was placed at the center of the enamel surface to ensure reproducibility. The evaluations were carried out in a location sheltered from outside luminosity. The measurements were taken in duplicate to improve accuracy. When the two readings were the same for the VITA Classical scale, the value obtained at the second reading was used. If the two readings did not match, another measurement was made to reach agreement between the two readings.

All the color analyses regarding the parameters of the VITA Classical scale and the Commission Internationale de L'éclairage (CIE) color coordinates were performed using a spectrophotometer, where L* represents lightness, a* represents the point on a red-green scale, and b* represents the point on a yellow-blue scale (CIELAB).

Clinical Phase: Bleaching and Staining Protocols

A week before the bleaching treatment began (run-in period) and throughout the entire treatment, the participants standardized the toothbrush (Colgate Slimsoft, Colgate-Palmolive, São Bernardo do Campo, Brazil) and dentifrice used (Colgate Maximum Anticaries Protection, 1500-ppm fluoride toothpaste, Colgate-Palmolive).

Figure 1. *Line 1: in-office bleaching (40% hydrogen peroxide); line 2: no bleaching (control); line 3: at-home bleaching (10% carbamide peroxide). Column 1: immersion in coffee; column 2: no immersion; column 3: immersion in cola solution.*

Nine enamel slabs were positioned in the appliance according to the treatments to be applied, randomly allocated in lines and columns (Figure 1). The devices were delivered to the volunteers early in the morning, together with instructions for daily use, for a total of 15 days. The volunteers were told to use the device throughout the day and night and remove it only for meals. When the device was removed from the oral cavity, it had to remain in a plastic box wrapped in damp gauze.[22] The device and the dental blocks had to be brushed twice a day with toothbrush and toothpaste, applied with 10 back-and-forth movements on top of each row.

After the first four hours of use (in which there occurred the formation of a pellicle of acquired biofilm),[29] the devices were collected by the researcher for the application of bleaching agents (Table 1). The enamel slabs on line 1 received the in-office bleaching treatment with 40% hydrogen peroxide (Opalescence Boost PF 40% [OPA40], Ultradent). The enamel slabs in line 2 received no bleaching agent (control). In line 3, the bleaching treatment was performed with 10% carbamide peroxide (Opalescence PF 10% [OPA10], Ultradent) (Figure 1). In applying the bleaching agents, 0.02 mL of each gel was applied to the surface of the dental enamel, taking care not to let the gel overflow and invade the neighboring enamel blocks. OPA40 was applied only on treatment days 1, 8, and 15; it had to remain on the surface of the enamel for 40 minutes. OPA10 was applied daily for 60 minutes, for a total of 15 days of treatment (except on weekends, when no bleaching

Table 1: *Bleaching Agents, Compositions, and Manufacturers*

Bleaching Treatment	Composition (Percentage in Weight)[a]	Mode of Use
OPA40, Opalescence Boost PF 40% (Ultradent South Jordan, UT, USA) BCRFX	Hydrogen peroxide (<40), sodium fluoride (1.1-1.5), potassium nitrate (<5), potassium hydroxide (<5)	40 min per session for three sessions with 1-wk intervals between sessions
OPA10, Opalescence PF10% (Ultradent) DO2J6	Carbamide peroxide (<20), polyacrylic acid (<10), sodium fluoride (0.25), sodium hydroxide (<5)	60 min per day for 15 d

[a] *According to the material safety data sheet of each product.*

treatment was performed). Removal of the bleaching agents was done initially with gauze, followed by washing with distilled water for 10 seconds.

After performing the bleaching treatments, the device was inserted into the oral cavity to be used by the research volunteer for two hours. After this time, the devices were again collected for immersion of the blocks into the staining solutions daily for a total of 15 days, except on weekends, where no spotting treatment was performed.

The enamel slabs from column 1 (Figure 1) were submitted to staining with coffee. The coffee was prepared with 50 mL of hot water and one teaspoon of soluble coffee (Nescafé Original, Nestlé, Araras, Brazil), according to the manufacturer's instructions. The enamel slabs were immersed into 30 mL of the coffee solution (considering a volume of 10 mL of solution per dental block) for 30 minutes daily, simulating the contact of the coffee with the dental structure for one day of consumption.[19,22] After immersion, the blocks were washed in distilled water for 10 seconds. The enamel slabs from column 2 were not submitted to any staining solution. Finally, the slabs from column 3 were submitted to the cola solution (Coca-Cola, Rio de Janeiro, Brazil) by immersing them in 30 mL of this cola (considering a volume of 10 mL of solution per dental block) for 30 minutes daily.

The pH for each bleaching agent and solution was obtained by using a pH meter (MS Tecnopon Special Equipment Ltd, Piracicaba, Brazil) and in triplicate. The corresponding pH values obtained were as follows: 6.64 for the at-home bleaching agent, 7.24 for the in-office bleaching agent, 2.51 for the cola, and 4.78 for the coffee.

At the end of 15 days, the intraoral device was collected on the morning following the last day of treatment. A representative figure comparing the *in situ* device before and after 15 days of use is presented in Figure 2. The enamel blocks were

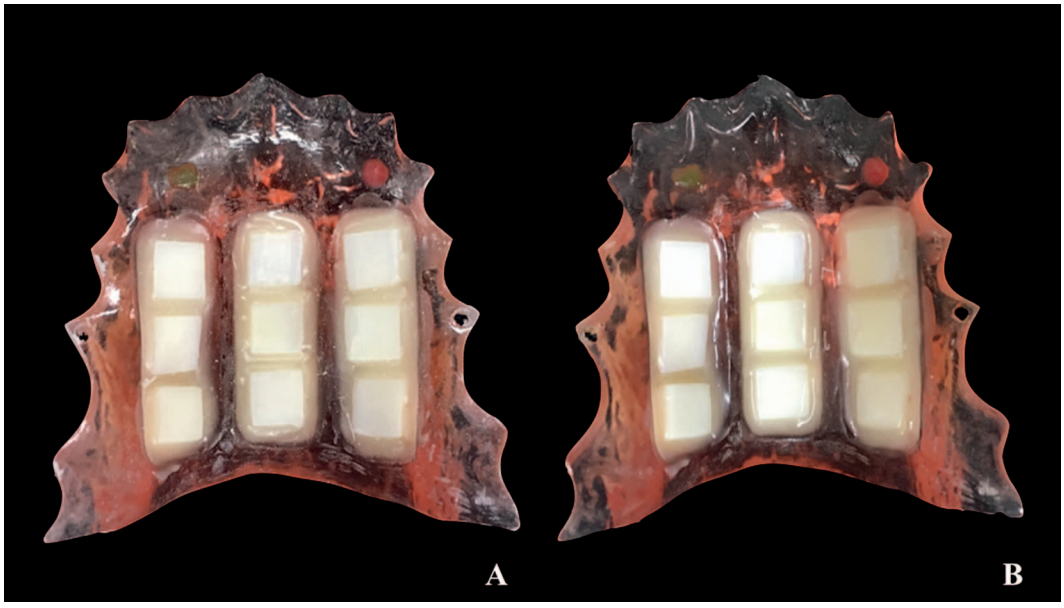

Figure 2. *Representative images of the* in situ *device before and after 15 days of use. (A):* In situ *device before insertion into the oral cavity. (B):* In situ *device after 15 days of use.*

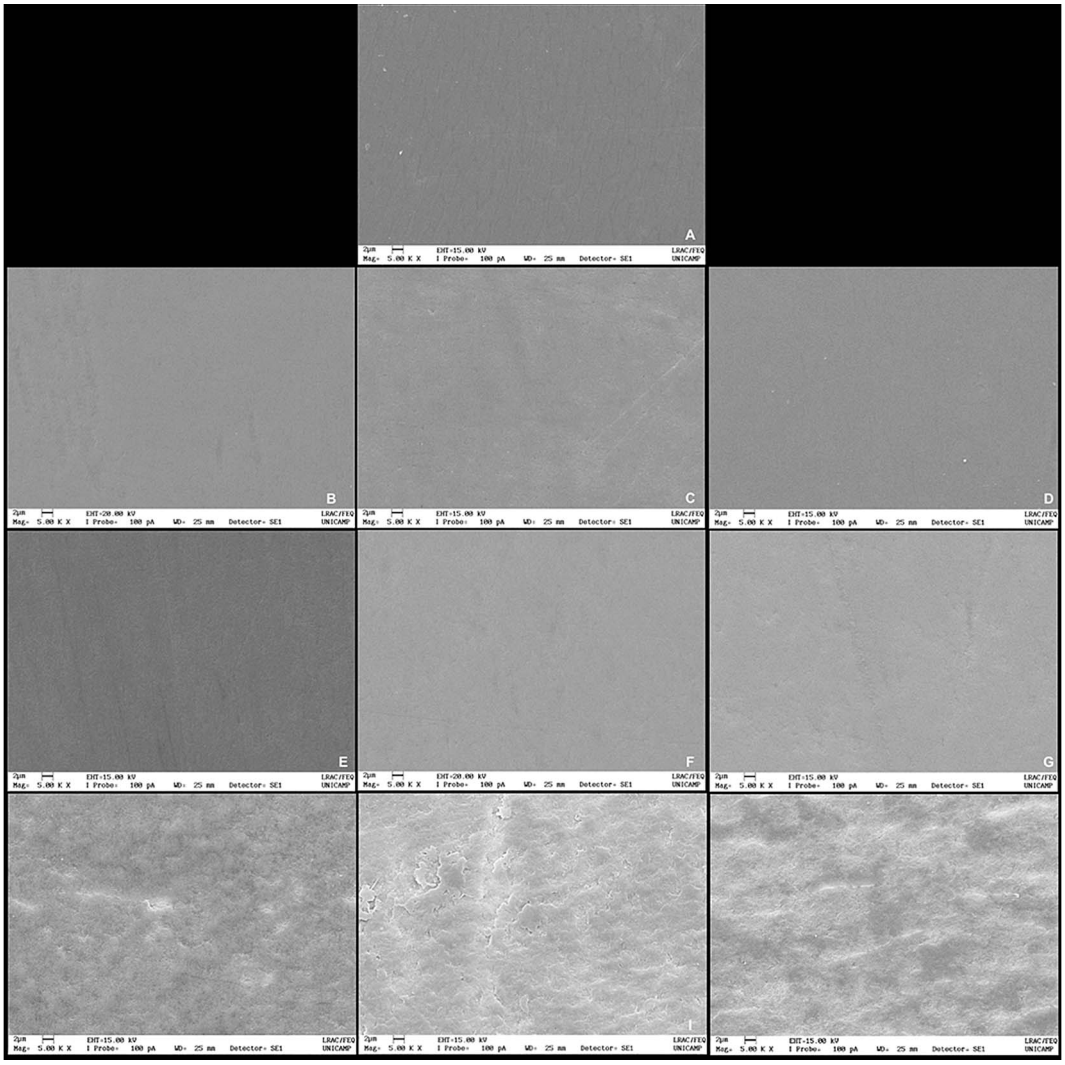

Figure 3. *Representative enamel surface micromorphological features. (A): Baseline. (B): Coffee + at-home bleaching. (C): Coffee + in-office bleaching. (D): Coffee + no bleaching. (E): No solution + at-home bleaching. (F): No solution + in-office bleaching. (G): No solution + no bleaching. (H): Cola solution + at-home bleaching. (I): Cola solution + in-office bleaching. (J): Cola solution + no bleaching.*

removed from the intraoral device and stored in Eppendorf tubes left in relative humidity for final analysis of microhardness, roughness, and color.

Micromorphological Analysis of the Enamel Surface

Three enamel blocks from each group (before and after each combination of bleaching treatments and staining solutions) were separated for analysis by scanning electronic microscope (SEM) (LEO Electron Microscopy, Oxford, England) to obtain representative images. They were submitted to gold sputtering. Images were taken at 5000× magnification. A single operator evaluated the enamel surface for the presence of erosion, irregularities, and depressions. The examiner was blinded during the SEM evaluation.

Statistical Analysis

The color of the tooth was obtained according to the VITA Classical scale and was converted into numbers according to numerical values previously established in the literature:[13] B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, and C4. Note that the smaller the numerical value, the brighter the tooth appears. The values of ΔL^*, Δa^*, and Δb^* were calculated by comparing the baseline to the after-treatment time period. After obtaining the values of ΔL^*, Δa^*, and Δb^* for each treatment, ΔE^* was calculated using the following mathematical formula: $\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$, where ΔE^* = the color change, ΔL^* = L*final – L*initial, Δa^* = a*final – a*initial, and Δb^* = b*final – b*initial. The color data in the VITA Classical scale and CIELAB

Table 2: *Microhardness Means (Standard Deviations) in kgf/mm² According to Staining Solution, Bleaching Agent, and Time Period*[a]

Time Period	Solution	Bleaching Agent		
		No Bleaching	At-Home	In-Office
Baseline	Coffee	370.56 (40.84) Aa	377.53 (43.73) Aa	395.13 (49.24) Aa
	Cola	396.00 (37.40) Aa	365.09 (36.94) Aa	375.93 (43.70) Aa
	No solution	367.40 (36.59) Aa	370.22 (48.73) Aa	370.13 (35.62) Aa
Final	Coffee	353.16 (98.86) Aa	364.69 (106.85) Aa	304.90 (85.90) Aa
	Cola	121.44 (78.10) Ab[b]	130.76 (77.71) Ab[b]	116.76 (90.31) Ab[b]
	No solution	398.98 (116.51) Aa	339.77 (91.74) Aa	358.80 (99.47) Aa

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions at each time period) differ among the time periods (p≤0.05).*
[b] *Differ significantly from the baseline in the same conditions of bleaching agent and solution (p≤0.05).*

system did not meet the assumptions of a parametric analysis and were analyzed by generalized linear models considering the study design.

The microhardness data met the assumptions of a parametric analysis. Roughness was determined by performing logarithmic transformation. Mixed-model methodology was applied for repeated measurements in a 3×3 (PROC mixed) factorial scheme for microhardness and roughness. All analyses were carried out using SAS software (version 9.2, SAS Institute Inc, Cary, NC, USA) considering a 5% level of significance.

RESULTS

There was no significant interaction among the factors of type of bleaching treatment, staining solution, and time period influencing microhardness ($p=0.1556$), but there was a significant effect resulting from the interaction between staining solution and time period ($p<0.0001$). Microhardness averages were not significantly different among the treatments at the baseline time. At the final time period (after the last bleaching session), the microhardness of the enamel submitted to staining with cola was significantly lower than that of the other groups ($p<0.0001$), highlighting a significant decrease in microhardness ($p<0.0001$) in relation to the initial time period regardless of whether bleaching was performed (Table 2).

There was no significant interaction among the factors of type of bleaching, staining solution, and time period influencing roughness values ($p=0.9112$), but there was a significant effect of these factors on the interaction between staining solution and time period ($p<0.0001$). At the beginning, there was no significant difference among the treatments. However, at the final time period, the roughness was greater for the enamel submitted to cola ($p<0.0001$) and increased significantly by the final time period regardless of whether bleaching was performed ($p<0.0001$) (Table 3).

There was a significant effect between staining solution and time period for the VITA Classical shade ($p<0.0001$) but no significant effect of the interaction among the factors of bleaching treatment, staining solution, and time period ($p=0.5684$). In the groups where no staining solution was used, a smaller color score was observed at the final vs the

Table 3: *Roughness Means (Standard Deviations) in μm According to Staining Solution, Bleaching Agent, and Time*[a]

Time Period	Solution	Bleaching Agent			
		No Bleaching	At-Home	In-Office	
Baseline	Coffee	0.04 (0.01)	0.04 (0.01)	0.03 (0.01)	a
	Cola	0.04 (0.01)	0.03 (0.01)	0.05 (0.02)	a
	No solution	0.04 (0.02)	0.04 (0.01)	0.03 (0.01)	a
		A	A	A	
Final	Coffee	0.05 (0.06)	0.04 (0.02)	0.04 (0.02)	b
	Cola	0.20 (0.05)[b]	0.18 (0.05)[b]	0.21 (0.06)[b]	a
	No solution	0.04 (0.01)	0.04 (0.01)	0.03 (0.01)	b
		A	A	A	

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions at each time period) differ among each time period (p≤0.05).*
[b] *Differ significantly from the baseline in the same conditions of bleaching agent and solution (p≤0.05).*

Table 4: *Means (Standard Deviations) of the VITA Classical Scores According to Staining Solution, Bleaching Agent, and Time Period*[a]

Time Period	Solution	Bleaching Agent			
		No Bleaching	At-Home	In-Office	
Baseline	Coffee	11.13 (1.55)	10.73 (2.66)	10.67 (2.61)	a
	Cola	10.33 (2.55)	10.20 (3.23)	10.33 (2.38)	a
	No solution	10.36 (2.59)	11.27 (1.71)	10.67 (3.58)	a
		A	A	A	
Final	Coffee	10.60 (3.20)	10.47 (2.77)	8.93 (4.20)	a
	Cola	10.79 (2.58)	11.07 (3.01)	9.47 (3.48)	a
	No solution	8.27 (3.03)[b]	8.93 (3.41)[b]	5.67 (3.85)[b]	b
		A	A	B	

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions for each time period) differ among each time period ($p \leq 0.05$).*
[b] *Differ significantly from the baseline in the same conditions of bleaching agent and solution ($p \leq 0.05$).*

initial time periods ($p<0.0001$). The color scores for OPA40 were significantly lower than those of the other treatments at the final time period ($p=0.0034$). The enamel color scores were lower at the final time period, when no staining solution was used ($p=0.0154$) (Table 4).

When in-office bleaching was performed and no staining solution was used, a greater increase in the ΔL value was observed than for at-home bleaching ($p=0.0444$). There was no significant difference between the bleaching agents when they were associated with the staining solutions. There was also no significant difference between the bleaching agents in regard to Δa ($p=0.8270$), and lower values were observed when no staining solution was used ($p=0.0010$). The Δb value also did not vary significantly between the bleaching agents ($p=0.3929$) but was significantly higher when cola was used ($p=0.0293$). In contrast, higher ΔE values were observed when cola was associated with the bleaching treatments ($p=0.0089$). Even when no staining solution was used, lower values were observed for the control and the at-home bleaching groups. Higher ΔE values were observed for in-office bleaching when no staining solution was used (Table 5), while lower values of ΔE were observed for the in-office associated with coffee immersion.

The SEM images (Figure 3) at baseline show that the enamel surface had a smooth and polished appearance (A). The groups submitted to the coffee solution and those not submitted to any solution (control) had the same characteristics of no erosion or porosity regardless of application or nonapplication of the bleaching agents (B to G). However, when the enamel was immersed in cola (H to J), the surface had a rough appearance, irregularities, and cracks regardless of whether the bleaching agent was applied.

DISCUSSION

The effectiveness of bleaching agents containing hydrogen peroxide and carbamide peroxide in high and low concentrations is influenced by the physical, mechanical, and micromorphological properties of the enamel subjected to beverages rich in pigments consumed during the bleaching technique. Even though laboratory studies have been carried out to evaluate this influence,[16,19-21] intraoral conditions cannot be reliably reproduced and must be analyzed with studies using *in situ* methodology.

When evaluating the microhardness of dental enamel not submitted to staining solutions, no significant changes were observed between the evaluation time periods with or without the bleaching agents. Both bleaching agents used have fluorides in their composition. This may have contributed to slowing the demineralization process[30] and enhancing enamel remineralization.[31,32] In addition, the manufacturer of the bleaching gels does not specify what thickening agent is used. Since carbopol (polycarboxylic acid)—present in the composition of many bleaching agents as a thickening agent—is known to cause enamel demineralization,[33,34] it may be suggested that both gels presented another component as the thickener. The pH values of the bleaching agents are also close to neutral (6.64 for the at-home agent and 7.24 for the in-office agent), thus contributing to balancing the demineralization and remineralization processes. Furthermore, the permanent presence of saliva—because this was a *in situ* study—has an enamel remineralizing action because it is supersaturated in minerals.[35-37] *In vitro*

Table 5: *Means (Standard Deviations) of the CIELAB Color Change According to Staining Solution, Bleaching Agent, and Time Period*[a]

Variable	Solution	Bleaching Agent		
		No Bleaching	At-Home	In-Office
ΔL^b	Coffee	0.39 (6.40) Aab	3.91 (6.69) Aa	2.33 (4.03) Ab
	Cola	−0.20 (4.03) Bb	0.67 (6.47) Aa	2.74 (5.44) Aab
	No solution	5.27 (5.56) ABa	3.47 (7.15) Ba	7.68 (5.20) Aa
Δa^c	Coffee	1.70 (2.28) Aa	1.54 (1.83) Aa	0.34 (1.89) Aa
	Cola	1.30 (2.01) Aa	1.63 (3.04) Aa	1.19 (2.52) Aa
	No solution	0.14 (0.91) Ab	−0.41 (2.23) Ab	−1.50 (2.60) Ab
Δb^d	Coffee	−0.27 (5.97) Aab	1.42 (5.49) Aab	−3.27 (6.41) Aab
	Cola	3.69 (4.44) Aa	2.52 (7.49) Aa	−0.33 (8.67) Aa
	No solution	−2.47 (3.48) Ab	−3.00 (3.78) Ab	−5.75 (6.25) Ab
ΔE^e	Coffee	7.77 (4.53) Aa	8.07 (5.44) Aa	7.83 (3.56) Ab
	Cola	6.40 (3.53) Ba	9.12 (5.32) Aa	9.89 (3.98) Aab
	No solution	7.44 (4.55) Ba	6.83 (6.56) Ba	11.47 (5.74) Aa

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions at each time period) differ among each time period ($p \leq 0.05$).*
[b] *p(bleaching)=0.0444; p(solution)=0.0886; p(bleaching vs solution)=0.0530.*
[c] *p(bleaching)=0.8270; p(solution)=0.0010; p(bleaching vs solution)=0.7010.*
[d] *p(bleaching)=0.3929; p(solution)=0.0293; p(bleaching vs solution)=0.2950.*
[e] *p(bleaching)=0.0089; p(solution)=0.7486; p(bleaching vs solution)=0.0718.*

studies have shown that even when artificial saliva solutions are used, bleaching gels with more acidic pH have been found to decrease enamel microhardness[35] and can negatively influence hardness values during bleaching.[38]

Even in the groups not submitted to any bleaching agent, the application of coffee did not promote any changes in enamel microhardness. However, the association of cola caused a decrease in microhardness in all the bleaching groups. Thus, the first null hypothesis was rejected, namely, that there are no differences in enamel microhardness between at-home and in-office dental bleaching submitted to staining solutions (coffee and cola) or not submitted to any solution. It is known that enamel exposed to solutions with a low pH for a certain period of time can present demineralization[37] or erosion.[40] Although the coffee solution presented a pH of 4.78, it was unable to cause demineralization in contact with the enamel for 30 minutes a day. Therefore, it can be suggested that, even with an acidic pH and with the same immersion time for coffee and cola, the demineralizing or erosive potential of coffee was not as harmful to the enamel as the effects caused by cola, which had a pH of 2.51. Among the soft-drink beverages, cola has the highest erosive potential[41,42] and requires more time for saliva to neutralize the acid medium.[42] Cola is also different from other soft drinks because of the phosphoric acid in its composition, which gives the beverage its acidity. Although there are a number of studies that show that cola causes a reduction in microhardness,[21,42] no studies were found that evaluated the influence of coffee on enamel microhardness. In the present study, even the constant presence of saliva and fluoride (from the fluoride toothpaste used for brushing the enamel blocks) was not enough to maintain or recover the initial microhardness values observed prior to immersion in cola.

The reduction in enamel microhardness when using cola was accompanied by increased roughness and by micromorphological changes similar to depressions and enamel erosion with or without use of the bleaching agents. Likewise, the second and the third null hypotheses were also rejected since differences were in fact observed in the enamel roughness and micromorphology between at-home and in-office dental bleaching submitted to staining solutions (coffee and cola) or not submitted to any solution. Other studies also reported that cola promotes greater enamel roughness[44,45] and leads to dental erosion.[41,46] These effects are duly explained by the acidic and erosive characteristics of the beverage, which raise roughness values to a threshold of about 0.2 μm.[47] These higher values may increase plaque accumulation. However, when neither coffee nor cola nor any bleaching agent was used, the roughness and micromorphology of the enamel were not altered, and the enamel surface looked smooth and polished, just like the bleached enamel surfaces observed in the *in situ* study by Zeczkowshi and others.[48] Although the literature

has reported that both at-home bleaching using 10% carbamide peroxide[7,49] and in-office bleaching[50] can change tooth roughness and promote erosion, the products currently available for dental bleaching have fewer detrimental effects due to the modernizing changes made in their composition over time. For example, the bleaching agents used today are less acidic than those available in the 1990s and in 2000, which had a pH from 4.3 to 5.2 for at-home use[51] and from 4.3 to 5.5 for in-office use.[5,10,32] Today's bleaching process has a shorter time protocol for using the agents in trays[52,53] and a modified thickener.[32,33] Although manufacturers do not declare the composition of bleaching agents in their entirety, it is known that the ingredients have been modified over time in response to the constant innovations and solutions that the research community has offered to manufacturers over time.

When no staining solution was used, the enamel had lower color scores for the VITA Classical scale, thus confirming that the bleaching treatments were effective in promoting the desired result. In regard to the bleaching agent used for at-home use, the color changed about three shades on the scale (from 11.27 before bleaching to 8.93 afterward), whereas the color change in the in-office treatment was five shades on average (from 10.67 to 5.67). Although these results may be related to the differences in the concentrations of the hydrogen peroxide agents used (10% carbamide peroxide for the at-home bleaching and 40% hydrogen peroxide for in-office) and the application time of only one hour per day for the at-home bleaching agent[52] (instead of overnight as recommended by the manufacturer), other studies have also shown that bleaching with carbamide peroxide in low concentrations[54,55] and hydrogen peroxide in high concentrations[56,57] has had satisfactory results. Furthermore, similar effectiveness between the techniques was reported in a systematic review,[58] but it did not take into consideration the variations in protocols (daily use time, number of bleaching sessions, and product concentration) of the bleaching techniques in the studies included.

Lighter tooth staining was also found in the present study when no gel was applied and no pigmented solution was used. Although all procedures were performed with utmost care to avoid contamination of the gel with blocks belonging to another group positioned alongside, it is believed that there may have been diffusion of the residual gel into the oral cavity, influencing the magnitude of ΔL^* and ΔE^*, which were high for this group despite meticulous removal of the gel. Because the devices were positioned on the palate, contact of the tongue with the surface of the enamel was unavoidable, and a small amount of gel residue could have diffused to the other blocks due to the low molecular weight of the free radicals released by the degradation of the peroxides. This is because peroxides can move freely between the structures of the substrates,[59] leading to the color changes observed in the nonbleached group caused by the proximity between the blocks. Although this approximation may have influenced the results for the no-bleaching and no-staining group, its effect cannot be avoided in the at-home and in-office bleaching groups submitted to the staining solutions together with other factors that may also explain the changes observed in the negative control. For example, although all care was taken to measure the colors correctly, the spectrophotometer may have been positioned incorrectly on the specimens, or the size of the enamel blocks may have been too small to accommodate the tip of the spectrophotometer, issues that will have to be dealt with more knowingly in future studies.

When analyzing the staining with cola vs coffee solutions, no differences were found based on the color analysis by the VITA Classical scale between before and after bleaching or between these solutions in the group that did not undergo bleaching. Thus, it may be suggested that, although the bleaching treatment was effective (as observed in the nonstained groups), the staining solutions used may have interfered in the final result of the treatment. Hence, the fourth null hypothesis was rejected since there were no differences in the enamel color change between at-home and in-office dental bleaching submitted to staining solutions (coffee and cola) or not submitted to any solution. In this respect, it is known that extrinsic staining is associated with the adsorption of pigments on the enamel surface, which can be removed efficiently with professional prophylaxis.[60] It has been reported that coffee and cola can stain teeth due to their dark coloration and acidic pH.[21,61] Low pH values may cause increased enamel permeability,[9] which may facilitate the penetration of the pigments during bleaching, thus interfering in the color results.[13,21] However, it has been found that the compounds that cause staining are constituted by chains of macromolecules, which barely permeate the enamel,[62] causing the pigments to be adsorbed on the surface of the enamel.

When analyzing the change in brightness (ΔL), the groups not subjected to bleaching or staining presented higher values than the group submitted

to staining with cola, which showed a reduction in luminosity. A more pronounced change in luminosity was found for teeth submitted to in-office bleaching without staining (greater luminosity) than for at-home bleaching without staining. This result could be related to the higher concentration of hydrogen peroxide contained in the bleach used in the in-office versus at-home treatments. In the latter, the bleach may require more time in contact with the teeth to produce a result with higher luminosity, as obtained by Briso and others[25] and Attin and others,[16] who associated different staining solutions (coffee, grape juice, and black tea) with 10% carbamide peroxide and observed higher values of brightness variation in the absence of staining. The present study also showed that the cola reduced the brightness of the enamel when no bleaching treatment was used, corroborating the findings by Pirolo and others,[63] who demonstrated the potential of cola to stain.

An analysis of the color change in the Δa variable, in the groups submitted to staining, indicated that more reddish pigments,[25] such as those present in coffee (brown pigment) and coca (caramel pigment), may not have actually been removed by different bleaching techniques but rather may have been adsorbed into the surface of the enamel. However, the most important color spectrum for whitening studies is the Δb variable since it is related to the color change of positive values, corresponding to colors from the yellow color band to the blue range, which presents negative values.[64] It was found that teeth became more yellow when the enamel blocks were subjected to cola solutions and showed intermediate yellowing values when submitted to coffee. When submitted to cola, the enamel blocks presented positive values, corresponding to the yellow color band regardless of having been submitted to a lightening process. When tooth enamel is exposed to an acidic substance, surface demineralization may occur, leaving the enamel more susceptible to pigment deposition, thereby explaining the results found.

Regarding color change (ΔE), values above 3.3 present clinically perceptible color changes and prove the effectiveness of the bleaching procedures.[65] ΔE values above 8 cause extremely perceptible changes.[66] In the group where the enamel blocks were not exposed to staining but were submitted to office bleaching, the value of ΔE was 11.47. This group was significantly different from the other nonstained groups, resulting in a desirable clinical response by the patients who completed the treatment. Even though all the other groups presented ΔE values above 3.3, indicating that all the groups presented clinically perceptible color changes after treatment, it still cannot be concluded that all the groups were affected by the bleach when considering each group (L*, a*, and b*) individually. This is because there is a "competitive" effect between the bleaching agents and the staining solutions that promotes bleaching (by degrading pigmented molecules) and darkening (by depositing dark pigments). Because ΔE values are the result of the interaction of all the parameters, they should not be evaluated separately; otherwise, there may be misinterpretation. Thus, it can be observed that the groups submitted to staining with cola had the following characteristics: 1) less alteration in luminosity than the bleached groups; 2) the teeth presented more red pigments than those not submitted to staining, whether or not they received the bleaching treatment; and 3) the teeth were more yellowish than those not submitted to staining, whether or not they received the bleaching treatment. In relation to staining with coffee, the lesser change in luminosity and the greater presence of reddish pigment may have influenced the ΔE values when associated with in-office bleaching. This can be explained by the daily immersion in coffee, whereas the in-office bleaching session was performed only once a week. Hence, this daily immersion influenced the color change, whereas the less frequent in-office dental bleaching decreased staining effectiveness, although a clinically perceptible color change was in fact achieved with in-office bleaching (ΔE value higher than 3.3).

Thus, considering the results for the VITA Classical scale and the L*, a*, and b* parameters, it can be concluded that the association of staining solutions to the bleaching treatment influences the color of the teeth during both at-home and in-office bleaching treatments and results in a less effective color change. In addition, cola also led to significant changes in microhardness, roughness, and micromorphology of the enamel. This may indicate that its consumption in everyday situations may also result in damage to the enamel surface.

CONCLUSIONS

Regardless of the bleaching treatment used (10% carbamide peroxide for at-home use and 40% hydrogen peroxide for in-office bleaching), there was a decrease in microhardness, an increase in roughness, and changes in the micromorphology of the cola-stained enamel. The effectiveness of the bleaching agents was higher in the absence of the

staining solutions (coffee or cola), and the in-office bleaching option presented a greater color change than at-home bleaching.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the São Leopoldo Mandic Research Institute Ethical Committee. The approval code for this study is 59850416.0.0000.5374.

Conflict of Interest

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

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Laboratory Research

Color Stability of Resin Cements Exposed to Aging

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Clinical Relevance

In certain preparations for indirect restorations, the cement line is exposed and unprotected by the ceramic. This condition leads to esthetic compromise over time.

SUMMARY

The aim of this study was to evaluate the color stability of light-cured and dual-cured resin cements after artificial accelerated aging. Ten specimens (6-mm diameter and 2-mm thickness) for each of five resin cements were prepared: GC (dual-cured cement, GCem), Vb (light-cured cement, Variolink II only the base), Vbc (dual-cured cement, Variolink II base with catalyst), VV (light-cured cement, Variolink Veneer), and FR (flowable resin composite, light cured). The samples were polished and stored in an accelerated artificial aging machine for 308 hours (160 klx), with cycles of 120 minutes under light and 60 minutes in the dark. All aging was carried out in distilled water at 37°C and light irradiation at 765 W/m^2. The samples were evaluated in a spectrophotometer before and after aging, and results were calculated according to CIEDE2000. The data were statistically analyzed (one-way analysis of variance and Tukey test, 95% confidence). The results of ΔE_{00} were statistically significant for the type of cement ($p<0.001$), with differences among tested groups. Variolink II (base only and base + catalyst) and the flowable resin were the cements with the lowest color variations after the artificial accelerated aging. Considering the values ΔE_{00} of acceptability and perceptibility, none of the tested cements showed acceptable values.

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INTRODUCTION

Ceramic laminates have emerged as an ideal esthetic treatment for anterior teeth. The treatment success

depends on (among other factors) the correct adhesive technique to bond the porcelain veneers.[1] Because of improvements in adhesives and resin cements over time, reliable and satisfactory results might be expected.

Cements with different polymerization mechanisms are available, having specific advantages and disadvantages.[2] Resin cements for veneers are normally activated by visible light. The major advantages of these systems are color stability and working time compared with chemically activated and dual-cured systems.[3] Dual-cured resin cements combine activation by light and chemical mechanisms; this type of activation leads to improved mechanical properties, such as increased flexural strength, elastic modulus, and hardness, when compared with other curing systems.[4] Another advantage for both the dual-cured and chemically-cured systems is the initiation of curing in regions where light cannot reach.[5,6] Dual-cured resin cements have a lower color stability compared with cements with only photoinitiators. The main disadvantage of this material might be the lack of color retention over time due to oxidation of tertiary amines in the formulation of dual cements.[7]

When dealing with very thin and/or translucent pieces of ceramic laminates, one should consider light-passing characteristics for choosing the resin cement. The color of employed resin cement influences the final color of the laminate after cementation,[8] although there is no agreement in the literature regarding the color stability of the final restoration. Ghavam and others showed that color changes in resin cement might affect the esthetics of ceramic laminates after aging,[2] while Turgut and Bagis reported no perceptible difference of color change in resin cements when underneath ceramic laminates.[9] Moreover, with minimally invasive preparations or cementing in unprepared surfaces, the termination region is usually exposed and cement is left unprotected by the ceramic, with such cement possibly being esthetically harmed over time.

Resin cements are subject to intrinsic and extrinsic staining factors. The intrinsic factors are those related to the materials composition, filler content, and type of activation. There are reports that triethylene glycol dimethacrylate is more susceptible to staining, with larger filler particles also influencing the discoloration of the material as they detach themselves from the organic matrix, resulting in a rougher surface that is more susceptible to staining.[9-11] Extrinsic factors are the adsorption and absorption of media, including food and beverage dyes.[10]

In addition to those considerations, the survival rate of veneers is about 94.4% after 12 years, with a low clinical failure rate (approximately 5.6%).[1] With few mechanical failures reported, veneer restorations will remain in service for a long time, and thus materials might be susceptible to aging. Marchionatti and others performed a clinical study that showed marginal discolorations after 24 months in 40% and 30% of veneers, cemented with light and dual-curing systems, respectively.[12] Thus, the objective of this study was to evaluate the color stability of light-cured and dual-cured resin cements exposed to accelerated artificial aging. The null hypothesis tested was that no difference in color variations will be detected considering the different types of resin cement tested under artificial aging.

METHODS AND MATERIALS

Five resin cements were used, as shown in Table 1. Ten disc-shaped specimens (diameter: 6 mm; height: 2 mm) were prepared from each material. Metal matrixes with Teflon tape were used for preparing specimens.

The top surfaces of all samples were irradiated for 60 seconds with a light-curing unit (Radii Cal LED, SDI, Victoria, Australia) operating on standard mode and emitting 1200 mW/cm^2 irradiance, measured by a radiometer (Demetron LED Radiometer, Kerr Corporation, Middleton, WI, USA).

The top surfaces of all specimens were polished using the complete sequence of a composite polishing system (Astropol, Ivoclar Vivadent, Schaan, Liechtenstein) from coarse to superfine. Each disk was used for 30 seconds with a handpiece rotating at approximately 10,000 rpm. This step was performed to remove the oxygen-rich surface layer and to flatten and polish the surfaces. The same operator polished all samples in random sequence. After polishing, the specimens were washed to remove any remaining debris.

Color was evaluated before and after accelerated photo-aging periods at 308 hours using specific aging equipment (SunTest CPS+, Atlas, Mount Prospect, IL, USA). The specimens were exposed to a filtered xenon lamp with an illuminance of 160 kilolux and irradiation of 765 W/m^2. Each 180-minute cycle consisted of 120 minutes exposed to a light followed by 60 minutes in the dark. The samples were stored in a container with distilled water at constant room temperature (37°C).[13-15]

Table 1: *Materials Used in the Study*[a]

Resin Cement	Group	Curing	Composition	Manufacturer
GCem	GC	Light-cured	Dimethacrylates, 4-META, phosphoric ester monomer, fluoro-aluminio-silicate glass, camphorquinone	GC Corporation, Tokyo, Japan
Variolink II	Vbc	Dual-curing	Bis-GMA, UDMA, TEGDMA, barium glass, ytterbium trifluoride, Ba-Al-fluorosilicate glass, spheroid mixed oxide, catalysts, stabilizers, and pigments	Ivoclar Vivadent AG, Schaan, Liechtenstein
Variolink II (only base)	Vb	Light-cured	Bis-GMA, UDMA, TEGDMA, ytterbium trifluoride, barium glass, ytterbium trifluoride, Ba-Al-fluorosilicate glass, spheroid mixed oxide, catalysts, stabilizers, and pigments	Ivoclar Vivadent AG
Variolink Veneer	VV	Light-cured	UDMA, TEGDMA, silicon dioxide, initiators, and stabilizers (Value O)	Ivoclar Vivadent AG
Grandio (flowable resin)	FR	Light-cured	BIS-GMA, TEGDMA, HEDMA, functionalized SiO_2 nanoparticles with glass ceramic particles[b]	Voco GmbH, Cuxhaven, Germany

Abbreviations: 4-META, 4-methacryloxyethyl trimellitic acid; Bis-GMA, bisphenol A diglycidyl ether dimethacryalate; HEDMA, hexane diol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.
[a] *Abbreviations used for group, type of polymerization, composition given by manufacturer, and the manufacturer.*
[b] *Morresi AL, D'Amario M, Monoco A, Rengo C, Grassi FR, & Capogreco M (2005) Effects of critical thermal cycling on the flexural strength of resin composites* Journal of Oral Science ***57(2)*** *137-143.*

The color measurements were performed with a spectrophotometer (CM-2600D, Konica Minolta, Ramsey, NJ, USA) according to the CIE-Lab (Commision Internationale de l'Eclairage, L*, a*, b*) coordinates with a D65 standard light source. The CIE L* parameter corresponds to the degree of lightness and darkness, whereas a* and b* coordinates correspond to red or green (+a* = red, −a* = green) and yellow or blue (+b* = yellow, −b* = blue), respectively. The colorimeter was calibrated with a standard white and black plate every 20 specimens. For the evaluation of the color of the specimens, the reading was performed under reflectance on a white background (L: 84.95; a: −0.38; b: 2.93), with ultraviolet component included.

The color difference was calculated using the CIEDE2000 (ΔE_{00}) formula[16,17]:

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

where $\Delta L'$, $\Delta C'$, and $\Delta H'$ are the differences in lightness, chroma, and hue for the samples (before and after aging) in CIEDE2000 and RT is a function (the so-called rotation function) that accounts for the interaction between chroma and hue differences in the blue region. Weighting functions, SL, SC, and SH, adjust the total color difference for variation in the location of the color difference pair in L, a, and b coordinates, and the parametric factors, KL, KC, and KH, are correction terms for experimental conditions.[16,17]

Statistical treatment was performed by descriptive statistics (mean values and standard deviations), one-way analysis of variance (ANOVA) and two-way repeated-measures ANOVA, and Tukey's test with 95% confidence in Minitab 17 software (Minitab Inc, State College, PA, USA). Data were previously checked and confirmed for normality and homogeneity using Kolmogorov-Smirnov and Levene's tests ($p>0.05$), respectively.

RESULTS

The results of ΔE_{00} are presented in Table 2. One-way ANOVA showed a statistically significant difference for the type of cement ($p<0.001$). Using the Tukey's test (95%), GCem and Variolink Veneer cement presented the highest color changes. Variolink II (only the base and base + catalyst) cement and flowable composite had the least variation in color after the artificial accelerated aging.

Table 3 shows the mean and standard deviation values for each resin cement before and after the artificial aging. Tukey's test was performed for each parameter of L, a and b. All cements showed statistical difference between values, considering the baseline and after aging data within each group. An exception should be mentioned for the flowable resin b parameter and for Variolink Base in a and b parameters. The greatest differences were detected for Variolink Veneer and GCem cement, in agreement with their respective ΔE_{00} results. When a and b parameters were observed, a huge difference was seen in the Variolink Veneer group. All materials increased their b parameter values after aging, except for Variolink Veneer.

Table 2: *Mean and Standard Deviation of ΔE_{00} for Each Group, Coefficient of Variation, and 95% Confidence Interval*[a]

ΔE_{00}	Mean ± SD	Coefficient of Variation	Confidence Interval
GC	5.051 ± 0.594[A]	23.37	4.458; 5.644
Vb	2.013 ± 0.504[B]	75.99	1.420; 2.606
VV	4.458 ± 1.521[A]	41.72	3.865; 5.051
Vbc	2.770 ± 0.970[B]	44.56	2.177; 3.363
FR	1.991 ± 0.687[B]	54.90	1.398; 2.584

Abbreviations: FR, Grandio (flowable resin); GC, GCem; Vb, Variolink II (only base); Vbc, Variolink II; VV, Variolink Veneer.
[a] *Tukey's test is represented by uppercase letters within the mean column; different letters represent a statistically significant difference among the groups.*

DISCUSSION

According to the results obtained from the experimental groups, the null hypothesis that groups would not present statistical differences for color change was rejected. Groups with samples made from Variolink II cement (only the base and base + catalyst) and flowable composite presented the least variation in color after being subjected to artificial accelerated aging.

Most studies use the CIELab color difference to evaluate their results, but CIEDE2000 was developed to correct the CIELab and to better determine color acceptability and perceptibility. This correction was determined between the computed or measured color and the perceived visual color for better application in a clinical context.[12,18]

The color difference $\Delta E_{00} = 1.8$ refers to 50:50% acceptability, and $\Delta E_{00} = 0.8$ refers to 50:50% perceptibility, according to Paravina and others.[19] Taking that into consideration, none of the cements used in this study presented results within that range. On the other hand, color variations should also be evaluated for the other parameters, such as ΔL, Δa, and Δb, as seen in Table 3. The variations in L, *a*, and *b* parameters agreed with the ΔE_{00} results. The *a* parameter shows the color variation between green (negative) and red (positive), indicating that Variolink Veneer was susceptible to a change in red color after aging. The *b* parameter represents the variation between blue (negative) and yellow (positive). As the Δb value for Variolink Veneer was smaller after aging, a decrease in yellow tone was observed. Kilinc and others found similar results when they used Nexus 2 (bisphenol A diglycidyl ether dimethacrylate and dimethacrylate) in dual-cured mode.[20] The authors blamed the bleaching effect of the ceramic veneer for the cement lightening but did not further explain why this occurred. In fact, the material itself suffered a bleaching effect; therefore, the highest color variation ΔE_{00} for Variolink Veneer should not be considered without a comprehensive examination of the other parameters. The bleaching effect may be related to a continuously delayed dark curing with further consumption of initiators. Moreover, residual molecules may be further excited under intense light of the aging procedure, which could intensify the bleaching effect. In general, overall color variation is not expected, but it is better when this variation does not lead to yellowing. This was the case in the present study and also in the study by Kilinc and others. Further studies need to address the reasons why this occurred.

Despite the observed different behavior in color variation, the compositions of Variolink II and Variolink Veneer are close, namely, inorganic filler, dimethacrylate, catalysts, stabilizers, and pig-

Table 3: *Mean and Standard Deviation of L, a, and b Parameters Before and After Aging*[a]

Group	Color Reading	L*	a*	b*
GC	Baseline	68.39 (0.90)c	6.36 (0.44)g	21.62 (0.58)b
	After aging	69.88 (1.13)a	2.72 (0.50)a	27.06 (2.39)a
VB	Baseline	71.80 (0.41)b	3.74 (0.33)ab	25.33 (1.45)a
	After aging	69.84 (0.72)a	3.35 (0.34)bc	26.22 (1.87)a
VV	Baseline	76.67 (1.23)f	−2.25 (0.56)e	21.76 (1.75)b
	After aging	74.68 (0.71)e	0.05 (0.32)f	15.65 (1.50)e
VBC	Baseline	71.54 (0.82)b	4.36 (0.45)cd	23.41 (1.06)b
	After aging	69.40 (1.03)a	4.91 (0.62)d	27.26 (2.13)a
FR	Baseline	71.46 (1.03)b	3.72 (0.49)bc	18.10 (1.13)c
	After aging	70.25 (0.54)a	2.95 (0.51)a	18.62 (1.86)c

Abbreviations: FR, Grandio (flowable resin); GC, GCem; Vb, Variolink II (only base); Vbc, Variolink II; VV, Variolink Veneer.
[a] *Tukey's test of each parameter is represented by lowercase letters in columns; different letters represent a statistically significant difference among the values.*

ments. A slight difference is presented in the amount of inorganic filler, with Variolink Veneer presenting approximately 65% compared with 73% in Variolink II.[21] Variolink II (only the base) can also be used in the light-activated mode. According to Nathanson and Banasr, less color variation is expected when only the base is used compared with the regular activating mode (base + catalyst).[22] This may be related to low concentrations of chemical components attached while using in light-sensitive mode only. The composition of the cement base presents aliphatic and aromatic amines, whereas the catalyst contains benzoyl peroxide, which reacts with the aromatic amines for chemical polymerization. Ghavam and others did not find significant differences between these two groups (only the base and catalyst + base),[2] because although tertiary amines do not come into contact with the benzoyl peroxide of the catalyst, the base contains both amine components.[12]

Chemically cured cements also have a tertiary amine that aids in their curing. The degradation of residual amines and oxidation of residual carbon double bonds culminate in forming yellow components.[9] Variolink Veneer has a reduced amount of amine in its formulation according to its manufacturer, which should generate better chemical and color stability, which is different from what was seen in this study.

The GCem cement is not indicated for cementation of ceramic laminates according to the manufacturer. This cement contains phosphoric acid ester monomer, and all the self-adhesive cements that contain hydrophilic acidic monomers, such as carboxylic or phosphoric acid, can result in high sorption and solubility of their polymers.[23] This fact can be related to the results of improper color stability found in this study.

Turgut and Bagis reported the size of the filler particles of composites with discoloration.[9] Most composites have large filler particles and are more susceptible to staining. However, this study used a nanoparticle resin with 80% inorganic filler (nanoparticles functionalized SiO_2 with particles of glass ceramics),[24] and this fact might explain its better behavior in terms of color stability. Large particles may debond from the resinous matrix, leaving a rough surface exposed to the oral media and increasing the susceptibility to staining. When smaller particles (nanoparticles) are used, even if they debond from the matrix, superficial flaws would be smaller.[10]

Specimen dimension does not relate to clinical characteristics when considering a possible cement line exposed to the oral environment. On the other hand, the specimen finishing protocol was defined as being the closest to a clinical sequence. The samples were polished with specific tips for clinical use with a handpiece. It was decided that the cement should be evaluated without a ceramic layer because color change would depend on many factors related to the ceramic, such as ceramic color, translucency, thickness of the restoration, sintering, number of firings, polishing, and so forth.[25] Moreover, the intention was to evaluate the color change for the cement alone, to simulate the aging of the cement line, since a ceramic laminate veneer would protect the resin cement and substrate from the influence of artificial accelerated aging.[26] The present results might overestimate the clinical results, since the surface exposed to aging was greater than clinical conditions. The present results are directly related to the behavior of the tested materials under the aging protocol.

There is no way to predict whether these results fully reflect clinical practice, since the aging was accelerated and the entire cement was exposed to light irradiation, and these are limitations of this study. The protocol used for aging puts the materials under conditions of higher temperatures, humidity, and irradiation by light; therefore, oxidation of the amino group used as an initiator in resin materials can occur and lead to some kind of color change in the resin cements used.[2,3] Artificial accelerated aging does not represent the behavior in the oral environment, but efforts are being made to standardize this method in research and to cause accelerated degradation in effective time to find answers about the studied materials.[26]

CONCLUSION

Although there are limitations in an *in vitro* study, flowable composite resin and Variolink II (only the base or base + catalyst) showed the lowest variation in color after being exposed to artificial accelerated aging. Considering the acceptability and perceptibility levels, none of the resin cement used showed acceptable values.

Conflict of Interest

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

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Effects of Pretreatment, Specimen Thickness, and Artificial Aging on Biaxial Flexural Strength of Two Types of Y-TZP Ceramics

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Clinical Relevance

Surface treatment of Y-TZP–based ceramics should be carried out with care to avoid unexpected negative effects on the material.

SUMMARY

Objectives: To evaluate the effects of surface treatment, specimen thickness, and aging on the biaxial flexural strength (BFS) of two types of yttria-stabilized, tetragonal zirconia polycrystal (Y-TZP) ceramics.

Methods and Materials: Disc-shaped specimens, 0.4 and 1.3 mm thick, made from hot isostatic pressed (Denzir) and non–hot isostatic pressed (ZirPlus) Y-TZP, were sandblasted, heat treated, and autoclaved. The surface topography was assessed in accordance with European Standard 623-624:2004 and the BFS tests in accordance with International Organization for Standardization Standard 6872:2008. For statistical analyses, one-way Shapiro-Wilk test, analysis of variance (*post hoc*: least significant differences), Mann-Whitney U-test, and Pearson correlation tests ($p<0.05$) were used.

Results: As delivered, the BFS of the 0.4-mm ZirPlus was >1.3-mm ZirPlus ($p<0.01$), and the BFS of the 0.4-mm Denzir was >1.3-mm Denzir ($p<0.001$). Sandblasting with 0.2 MPa reduced the BFS of the ZirPlus and Denzir discs ($p<0.01$), whereas sandblasting with 0.6 MPa increased the BFS of the 0.4-mm Denzir ($p<0.001$) and reduced the BFS of the 0.4-mm ZirPlus ($p<0.05$). Heat treatment significantly reduced the BFS of all the groups except for the 0.6 MPa sandblasted 0.4-mm ZirPlus. Autoclaving reduced the BFS of the as-delivered ZirPlus and Denzir specimens ($p<0.001$), whereas autoclaving the 0.6 MPa sandblasted and heat-treated specimens had no effect ($p>0.05$) on the BFS. The 0.6 MPa sandblasted, heat-treated, and autoclaved 0.4-mm Denzir exhibited higher BFS than the 0.6 MPa sandblasted, heat-treated, and autoclaved 0.4-mm ZirPlus ($p<0.05$).

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Conclusions: Thickness and surface treatment of Y-TZP–based ceramics should be considered since those factors could influence the BFS of the material.

INTRODUCTION

Zirconia was introduced into dentistry at the beginning of the 1990s, and various types of zirconia systems are currently available as biomaterials.[1-3] The zirconia (ZrO_2) ceramic is polymorphic and exists in three different phases—monoclinic, tetragonal, and cubic[4]—and compared with alumina and other dental ceramics has higher flexural strength and fracture resistance, making the ZrO_2-ceramic of particular interest as a material for dental cores/substructures.[1-3] In addition, ZrO_2 ceramics are opaque and have a relatively small particle size, which has been said to allow the possibility of minimally invasive restorations and the masking of discolored underlying tooth structure.[5]

Today, zirconia restorations for dental application are manufactured mainly of yttria tetragonal zirconia polycrystals (Y-TZP) using computer-aided design/computer-aided manufacturing (CAD/CAM) techniques,[1,3] and the restorations can be processed with hard or soft machining.[5-7] Hard machining uses sintered Y-TZP blocks subjected to the process of hot isostatic pressing (HIPing), which has been said to effectively reduce porosity and possibly improve the mechanical properties of the material.[8] Soft machining uses mainly Y-TZP presintered prefabricated blocks; an enlarged core/substructure for a crown or fixed partial denture (FPD) is machined from the presintered block and subsequently sintered to the desired dimensions before veneering.[6,7] The recommended thickness of conventional cores/frameworks made of Y-TZP ceramics has been said to have a usual range of between 0.3 and 0.6 mm.[9]

To improve the esthetic appearance, the Y-TZP cores/frameworks are usually layered with feldspar-based or glass ceramics. During the veneering process, the cores/frameworks are subjected to heat treatment up to around 900°C-1000°C, and it has been shown that heat treatment, like veneering, significantly affects the strength of zirconia.[10-12]

Various other mechanical processes, such as grinding, milling, and sandblasting, could affect the properties of zirconia dental restorations.[10-15] However, conflicting results concerning the effects of grinding/machining on the strength of zirconia ceramics have been reported.[13-15] For example, some studies have shown that grinding/machining can reduce the strength of zirconia,[13,15] whereas other studies show that grinding/machining increases the strength.[14,16] Moreover, surface treatments such as sandblasting have been shown to increase the biaxial flexural strength (BFS) of zirconia.[13,15] In addition, an earlier study demonstrated that reduced thickness of HIPed Y-TZP copies could affect the fracture resistance of veneered stylized all-ceramic crowns.[17]

Since the thickness of the material in dental cores/substructures is important and various surface and heat treatments are usually inevitable steps in the manufacturing and veneering processes of dental ceramic restorations, the aim of the present study was to examine the effect on BFS of HIPed and unHIPed Y-TZP ceramics of 1) specimen thickness, 2) heat treatment, 3) sandblasting using various pressure, and 4) artificial aging.

METHODS AND MATERIALS

The materials studied were unHIPed Y-TZP (ZirPlus, Cad.esthetics AB, Skellefteå, Sweden) and HIPed Y-TZP (Denzir, Cad.esthetics AB) ceramics (Figure 1a,b). The composition of the two types of ceramics was identical.

Preparation of Specimens

Two hundred disc-shaped (13-mm diameter) specimens were CAD/CAM manufactured from prefabricated blanks using the Cad.esthetics software system (Cad.esthetics AB). The ZirPlus and Denzir specimens were made in two different thicknesses: 0.4 and 1.3 mm. Means ± SD of the thickness of the Denzir discs (n=20) and the ZirPlus discs (n=20) were 1.3 ± 0.02 mm. Corresponding figures for the ZirPlus (n=80) and Denzir discs (n=80) were 0.4 ± 0.03 and 0.4 ± 0.05 mm, respectively. Figure 1a,b summarizes the various groups of the ZirPlus and Denzir specimens studied. The reason why these thicknesses (1.3 and 0.4 mm) were chosen was because the International Organization for Standardization (ISO) Standard 6872:2008[18] states that the thickness of the specimen should be 1.2 ± 0.2 mm, whereas the thickness of dental zirconia cores often is between 0.3 and 0.6 mm.[9,17]

Ten specimens of each type and thickness of the Y-TZP ceramic were studied as delivered, that is, directly after machining. In addition, 10 specimens of each thickness and type of Y-TZP were heat treated before testing in a similar way to veneering with a feldspar-based porcelain (VITA VM9, VITA Zahnfabrik, Bad Säckingen, Germany).[19] To study the effect of surface treatment and aging, before

Fig.1a

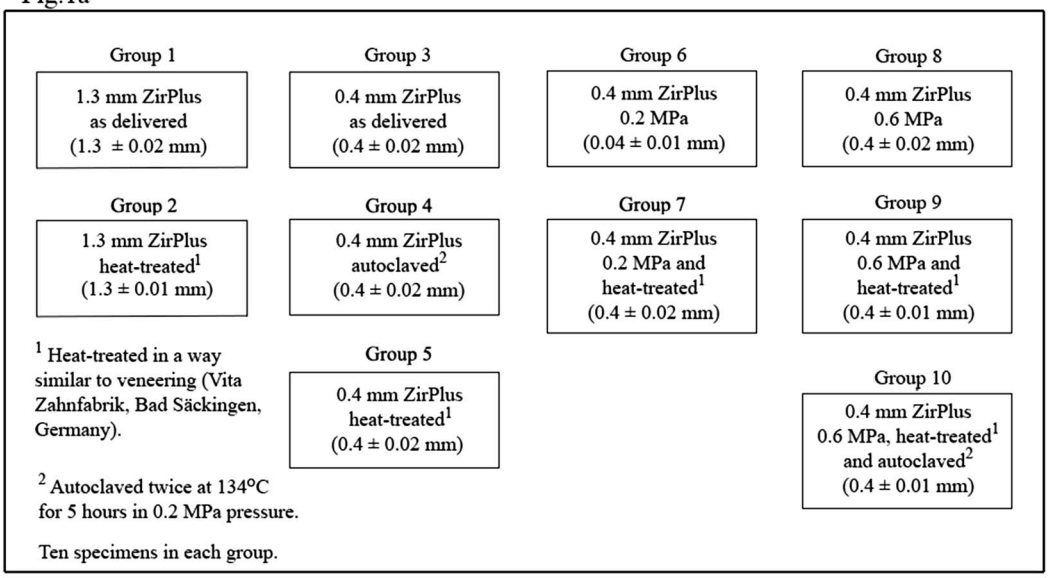

Fig. 1b

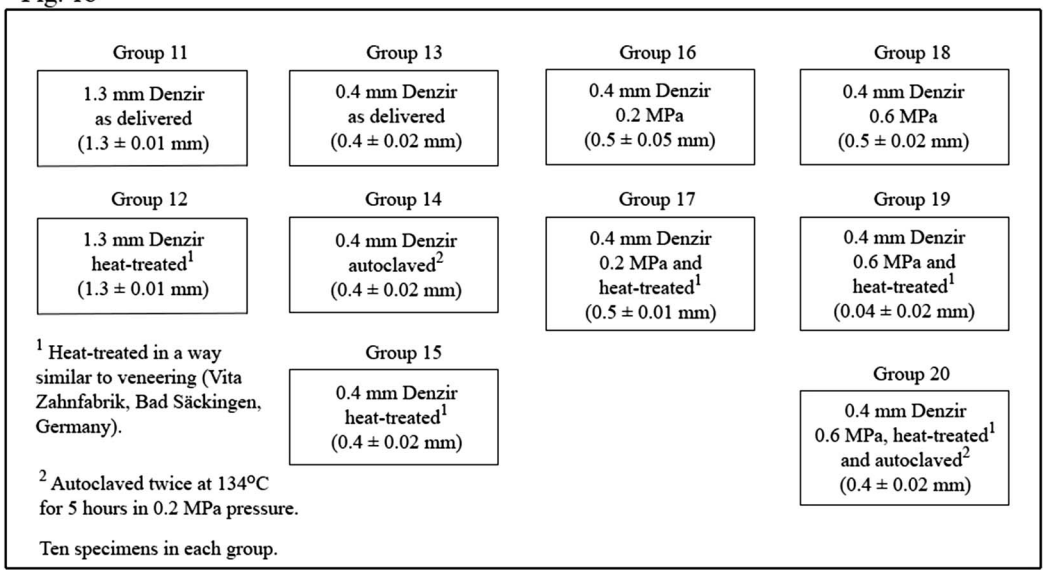

Figure 1a,b. *Flowchart of the ZirPlus and Denzir test samples. Means ± SD of the thickness of each group of the ZirPlus and Denzir discs studied are shown in parentheses. Ten specimens in each group.*

testing, the tensile surface of 50 discs of the 0.4-mm ZirPlus and 50 discs of the 0.4-mm Denzir discs were sandblasted (Basic Quattro, Renfert GmbH, Hitzingen, Gemany) for 90 seconds with 110 μm Al_2O_3 at a distance of ~2 mm between the surface of the disc and the nozzle (Basic Quattro). Of these 100 discs, the tensile surfaces of 20 of the 0.4-mm ZirPlus discs and 20 of the 0.4-mm Denzir discs were sandblasted with 0.2 MPa, and the tensile surfaces of 30 of the 0.4-mm ZirPlus discs and 30 of the 0.4-mm Denzir discs were sandblasted with 0.6 MPa (Figure 1a,b). Thereafter, 10 discs of each type were subjected to heat treatment similar to veneering (VITA VM9),[19] and 10 discs of each type of the as-delivered specimens were autoclaved (Kebo Lab AB, Stockholm, Sweden) twice for five hours at 134°C in 0.2 MPa pressure.[20] In addition, 10 discs of each type of the sandblasted 0.4-mm discs were heat treated in a way similar to veneering (VITA VM9)[19] and autoclaved twice for five hours at 134°C in 0.2 MPa pressure before testing (Figure 1a,b).[20] Between the two autoclave treatments, the autoclaved specimens were stored at room temperature for 16 hours.

Determining the Surface Roughness

Using a measuring profilometer (Taylor/Hobson Precision Form Taylor Surf 50, Taylor Hobson, Warrenville, IL, USA), the arithmetical mean deviation of the assessed surface roughness (*Ra*), the maximum profile valley depth (*Rv*), and the maximum height of the roughness (*Rz*) of the surfaces intended to be subject to tensile stress during the testing were measured; the cutoff length was 0.8 mm, evaluation length 4 mm, stylus speed 0.5 mm/s, and stylus tip radius 2 μm, in accordance with European Standard 623-624:2004.[21] Before testing, the specimens were cleaned ultrasonically (Bransonic B221, Bransonic Ultrasonic Co, Danbury, CT, USA) for five minutes in tap water, rinsed in distilled water, and air-dried. Five parallel scans were made at randomly selected locations in the center of the specimen on each measured surface. All measurements were recorded by one operator.

Test of Biaxial Flexural Strength

A universal testing machine (Tinius Olsen H10K-T, Horsham, PA, USA) was used for the BFS test, and the test setup was in accordance with ISO 6872:2008.[18] The support area for the specimens comprised three symmetrically placed steel balls, 3.2 mm in diameter, positioned 120° apart creating a circle with a diameter of 10 mm. The disc-shaped specimens were placed on the supporting balls, and the load was applied at the center of the specimen with a flat punch, 1.6 mm in diameter, at a crosshead speed of 1 mm/min. The sandblasted surface of the specimens was placed in tension; that is, the sandblasted specimens were placed in the sample holder with the sandblasted surface face-down bearing on the three stainless-steel supporting balls.

The load in newtons (N) required to fracture the specimens was automatically recorded, and the BFS was calculated using the following equations:

$$S = -0.2387\frac{P(X-Y)}{d^2} \quad (1)$$

where S is the maximum tensile stress (MPa) and P is the load at fracture (N) and

$$X = (1+v)1n(r_2/r_3)^2 + [(1-v)/2](r_2/r_3)^2 \quad (2)$$

$$Y = (1+v)[1+1n(r_1/r_3)^2] + (1-v)(r_1/r_3)^2 \quad (3)$$

where ν is the Poisson ratio, r_1 is the radius of the support circle, r_2 is the radius of the loaded area (mm), r_3 is the radius of the specimen (mm), and d is the specimen thickness (mm).

In the current study, the Poisson ratio (ν) = 0.25, $r_1 = 5$ mm, $r_2 = 0.8$ mm, and $r_3 = 6.5$ mm, was used. The thickness of the fractured surface of each specimen was measured using a measuring microscope (Leitz UWM-Dig-S, Ernst Leitz GmbH, Wetzlar, Germany) at 20× magnification at three selected measuring points: two points in the outer borders and one point in the center of the fractured discs. For each specimen, the mean value of the three measurements for each specimen was then used as the value for d in equation 1.

Statistical Analysis

The Shapiro-Wilk test was used to test normal distribution of the data. The BFS data were statistically analyzed using one-way analysis of variance supplemented with least significant differences *post hoc* tests ($p<0.05$). As the data of the surface roughness did not meet the normality requirement the Mann-Whitney U-test, a nonparametric test was used ($p<0.05$) to statistically analyze the *Ra*, *Rz*, and *Rv* values. The Pearson correlation test ($p<0.05$) was used to correlate the determined BFS with the surface roughness, that is, the determined *Ra*, *Rz*, and *Rv* values for each type of group. All data were analyzed using SPSS version 24 statistical software (Statistical Package for Social Science, SPSS Inc, Chicago, IL, USA).

RESULTS

Figure 2 presents the BFS of all the specimens studied, and Tables 1 and 2 present the statistical analyses of the BFS. Tables 3 through 5 show the arithmetical mean deviation and statistical analyses of the assessed roughness (*Ra*), the maximum valley depth (*Rv*), and the maximum height of the roughness (*Rz*).

Effects of Specimen Thickness, Heat Treatment, Sandblasting, and Autoclaving on the BFS

As delivered, the BFS of the 0.4-mm ZirPlus discs was significantly superior to the 1.3-mm ZirPlus discs ($p<0.01$), and the BFS of the 0.4-mm Denzir was significantly superior ($p<0.001$) to the 1.3-mm Denzir (Figure 2; Table 1). Sandblasting with 0.2 MPa reduced the BFS of the 0.4-mm ZirPlus and Denzir as-delivered discs ($p=0.000$), whereas sandblasting with 0.6 MPa increased the BFS of the 0.4-mm Denzir ($p<0.001$) and reduced the BFS of the

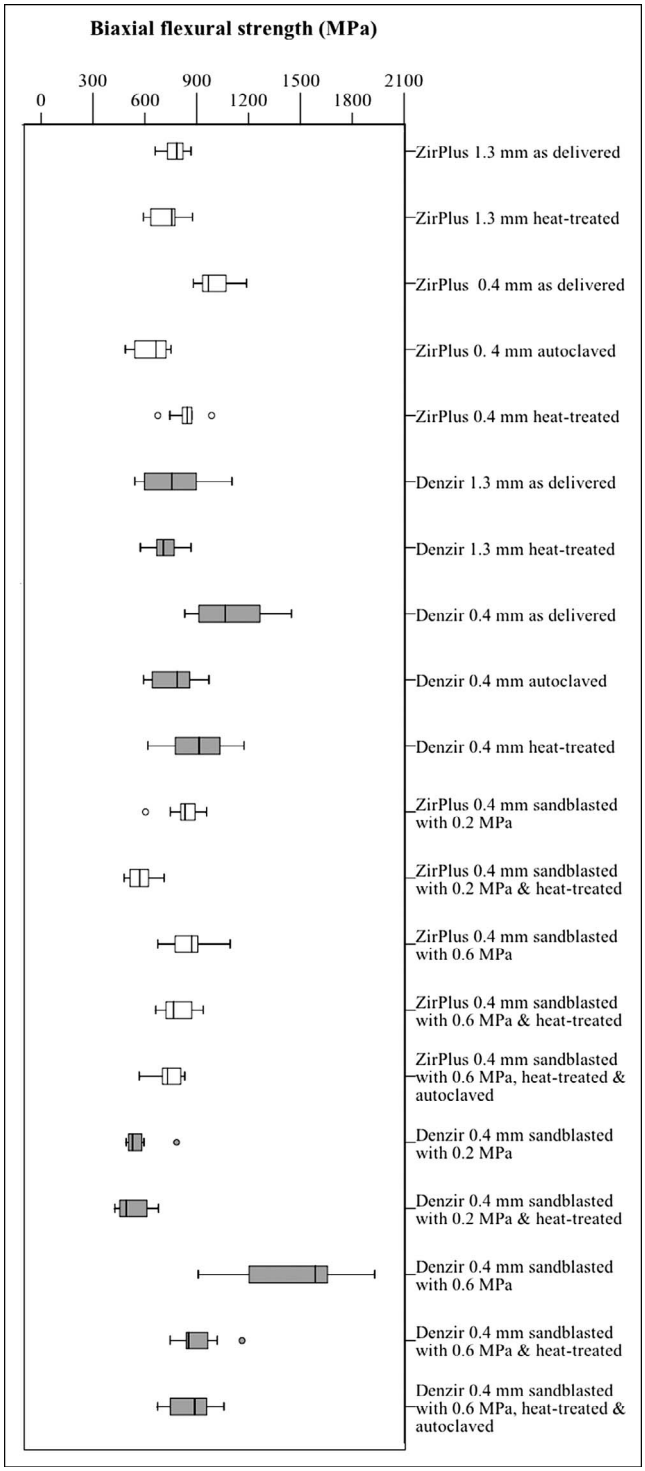

Figure 2. *Box plot of the biaxial flexural strength (MPa) of all the various groups studied. Ten specimens in each test group. Data are presented as medians and first and third quartiles. The median is presented by a horizontal line within the box. The maximum and minimum values are illustrated via the upper and lower strokes. All the sandblasted surfaces of the specimens were placed in tension. White boxes: ZirPlus specimens. Gray boxes: Denzir specimens.*

0.4-mm ZirPlus ($p<0.05$). Heat treatment significantly reduced the BFS of all the groups except for the 0.6 MPa sandblasted 0.4-mm ZirPlus. Autoclaving reduced the BFS of the as-delivered ZirPlus and Denzir specimens ($p=0.000$), whereas autoclaving the 0.6 MPa sandblasted and heat-treated 0.4-mm specimens had no effect ($p>0.05$) on the BFS (Figure 2; Table 2). The 0.6 MPa sandblasted, heat-treated, and autoclaved 0.4-mm Denzir exhibited significantly ($p<0.05$) higher BFS than the 0.6 MPa sandblasted, heat-treated, and autoclaved 0.4-mm ZirPlus (Figure 2; Table 2).

Surface Roughness and the Pearson Correlation Test

Of all the groups studied, the 0.6 MPa sandblasted ZirPlus discs exhibited the highest *Ra*, *Rv*, and *Rz* values (Tables 3-5). For the ZirPlus specimens, the Pearson correlation test revealed no statistically significant ($p>0.05$) correlations except between *Rv* and BFS of one of the 0.6 MPa sandblasted, heat-treated, and autoclaved 0.4-mm ZirPlus discs ($r=0.658$, $p=0.039$). For the Denzir specimens, the Pearson correlation test showed no statistically significant ($p>0.05$) correlations except for one specimen: one of the heat-treated 0.4-mm Denzir discs exhibited *Ra* and BFS ($r=-0.721$, $p=0.019$), *Rv* and BFS ($r=-0.776$, $p=0.008$), and *Rz* and BFS ($r=-0.731$, $p=0.016$).

DISCUSSION

Effects of Specimen Thickness

Today, minimally invasive restorations with high-level-esthetic appearance have become available for dental restorations, mainly because of the introduction of oxide-based ceramics,[1-3,10,17] and it has been stated that the small particle size of zirconia makes it possible to produce thin ceramic dental restorations.[5] However, the quality of zirconia can be affected, for example, by the grain size, type of stabilizing oxide, heat treatment, manufacturing process, and composition,[8,12,17,22-24] and the introduction of zirconia into dentistry has led to a number of questions about the proper handling of zirconia for dental applications. For example, in an earlier study,[17] stylized, veneered ceramic crowns with cores made of HIPed Y-TZP, 0.2 mm thick and placed on a slice-formed preparation, exhibited superior fracture strength compared to the values reported in a similar study of HIPed Y-TZP ceramic crowns with 0.5-mm cores placed on a circumferential chamfer.[10] In these previous studies,[10,17] it was proposed that the monoclinic phase/tetragonal phase

Table 1: *Summary of the Statistical Analysis (Analysis of Variance Supplemented With Least Significant Differences* Post Hoc *Test) of the Biaxial Flexural Strength of the As-Delivered and Heat-Treated ZirPlus and Denzir Specimens (10 Specimens in Each Group)*[a]

Specimen	A	B	C	D	E	F	G	H	I	J
A. ZirPlus, 1.3 mm, as delivered										
B. ZirPlus, 1.3 mm, heat treated	0.407									
C. ZirPlus, 0.4 mm, as delivered	0.000	0.000								
D. ZirPlus, 0.4 mm, autoclaved	0.029	0.174	0.000							
E. ZirPlus, 0.4 mm, heat treated	0.204	0.037	0.026	0.001						
F. Denzir, 1.3 mm, as delivered	0.840	0.530	0.000	0.048	0.141					
G. Denzir, 1.3 mm, heat treated	0.399	0.988	0.000	0.178	0.035	0.520				
H. Denzir, 0.4 mm, as delivered	0.000	0.000	0.090	0.000	0.000	0.000	0.000			
I. Denzir, 0.4 mm, autoclaved	0.877	0.500	0.000	0.043	0.155	0.963	0.490	0.000		
J. Denzir, 0.4 mm, heat treated	0.053	0.006	0.118	0.000	0.503	0.033	0.006	0.001	0.037	

[a] *Statistical differences (*$p<0.05$*). The values in the table refer to the* p*-values obtained.*

distribution in the Y-TZP core could have influenced the outcome.

Similarly, in the present study the BFS of the as-delivered 0.4-mm Denzir discs, that is, before a heat treatment similar to veneering, was significantly superior to the as-delivered 1.3-mm Denzir discs, and the BFS of the as-delivered 0.4-mm ZirPlus discs was superior to that of the as-delivered 1.3-mm ZirPlus discs (Figure 2; Table 1). That is, thinner specimens exhibited significantly ($p=0.000$) higher BFS than thicker specimens of a similar type of material. One possible explanation for thinner discs made of Y-TZP ceramics resulting in higher BFS could be the phase transformation t → m created on the surface because of the machining process. A monoclinic layer could be detected on ground/machined surfaces of Y-TZP ceramic,[25] and a proportionally thicker monoclinic layer may be created on thinner specimens than on thicker specimens. Since the monoclinic layer has ~3%-4% volume expansion compared to the tetragonal phase, the compressive layer formed at the surface by this layer could have resulted in the thinner specimens having a higher flexural strength than the thicker specimens.[13] It has been reported in a study by Ramos and others[25] that higher monoclinic content could be detected on ground zirconia surfaces compared to nonground surfaces and that subsequent heat treatment produced reversed transformation.[26] On the other hand, in a previous study of magnesia-stabilized zirconia (Mg-PSZ), the various specimen thicknesses, that is, 0.4-mm and 1.3-mm discs, did not influence the BFS.[2] However, it has been stated that phase transformation toughening is less in Mg-PSZ than in Y-TZP,[5,27,28] but it was also seen in the previous study of Mg-PSZ[2] that sandblasting and heat treatment could affect the strength of Mg-PSZ specimens. Another reason that the

Table 2: *Summary of the Statistical Analysis (Analysis of Variance Supplemented With Least Significant Differences* Post Hoc *Test) of the Biaxial Flexural Strength of the 0.2 and 0.6 MPa Sandblasted and Autoclaved ZirPlus and Denzir Specimens (10 Specimens in Each Group)*[a]

Specimen	K	L	M	N	O	P	Q	R	S	T
K. ZirPlus, 0.4 mm, 0.2 MPa										
L. ZirPlus, 0.4 mm, 0.2 MPa, heat treated	0.000									
M. ZirPlus, 0.4 mm, 0.6 MPa	0.575	0.000								
N. ZirPlus, 0.4 mm, 0.6 MPa, heat treated	0.521	0.001	0.229							
O. ZirPlus, 0.4 mm, 0.6 MPa, heat treated and autoclaved	0.125	0.013	0.037	0.370						
P. Denzir, 0.4 mm, 0.2 MPa	0.000	0.771	0.000	0.000	0.006					
Q. Denzir, 0.4 mm, 0.2 MPa, heat treated	0.000	0.393	0.000	0.000	0.001	0.573				
R. Denzir, 0.4 mm, 0.6 MPa	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
S. Denzir, 0.4 mm, 0.6 MPa, heat treated	0.289	0.000	0.617	0.090	0.010	0.000	0.000	0.000		
T. Denzir, 0.4 mm, 0.6 MPa, heat treated and autoclaved	0.544	0.000	0.963	0.212	0.033	0.000	0.000	0.000	0.650	

[a] *Statistical differences (*$p<0.05$*). The values in the table refer to the* p*-values obtained.*

Table 3: *Arithmetic Mean Deviation of the Assessed Profile (*Ra*) of the ZirPlus and Denzir Specimens (μm)*[a]

Specimen	ZirPlus (Mean±SD)	Denzir (Mean±SD)
1.32 mm, as delivered	0.3 ± 0.1 A	0.3 ± 0.1 A, B, E
1.32 mm, heat treated	0.3 ± 0.2 A, B	0.2 ± 0.1 C, H
0.4 mm, as delivered	0.2 ± 0.1 C	0.2 ± 0.1 C, K
0.4 mm, autoclaved	0.4 ± 0.1 D	0.4 ± 0.2 D
0.4 mm, heat treated	0.3 ± 0.2 A, B, C, E	0.2 ± 0.1 C, E, H, K
0.4 mm, 0.2 MPa	0.5 ± 0.2 F	0.6 ± 0.2 I
0.4 mm, 0.2 MPa, heat treated	0.5 ± 0.1 F, G	0.6 ± 0.1 G, I
0.4 mm, 0.6 MPa	1.3 ± 0.2	0.9 ± 0.2 J, L
0.4 mm, 0.6 MPa, heat treated	1.2 ± 0.3	0.9 ± 0.2 J
0.4 mm, 0.6 MPa, heat treated and autoclaved	1.4 ± 0.2	0.8 ± 0.1 L

[a] *Identical letters indicate absence of statistically significant difference (*$p > 0.05$*). No letter indicates statistically significant difference compared with all the other groups in respective table (*$p < 0.05$*). n = 10 in each group (Mann-Whitney U-tests).*

specimen thickness affected the strength of the Y-TZP specimens in the present study could be that the volumes between the 0.4-mm and the 1.3-mm specimens were different. That is, when the volume of the specimens increases, it will be more likely to hit strength-limiting defects in the ceramics, which could decrease the mean strength, that is, the effect of volume scaling.[29] Thus, when evaluating the results of, among others, the BFS tests, the thickness of the zirconia samples should be taken into consideration.

Effects of Surface Treatment

During the manufacturing processes of dental restorations, the surfaces of copies/frameworks are subjected to mechanical treatments, such as grinding, polishing, and/or sandblasting,[30,31] and previous studies show that the effects on the mechanical properties of such surface treatments and heat treatment of zirconia can vary.[13,32-34]

In order to mimic surface treatments in the present study, one of the flat surfaces of the 0.4-mm disc-shaped specimens was subjected to sandblasting using two different pressures: 0.2 MPa and 0.6 MPa. Sandblasting the 0.4-mm ZirPlus and 0.4-mm Denzir discs with 0.2 MPa significantly reduced BFS compared to the as-delivered specimens, whereas when a pressure of 0.6 MPa was used, the BFS of the 0.4-mm Denzir discs was significantly superior to the as-delivered discs, while the BFS of the 0.4-mm ZirPlus discs was reduced compared to the as-delivered discs. Possible explanations could be that 0.2 MPa sandblasting mimicked polishing rather than grinding of the specimen surfaces and that 0.6 MPa sandblasting caused a rougher surface of the ZirPlus discs than of the Denzir discs (Tables 3-5). It has also been demonstrated previously that surface treatments of zirconia could produce a variety of effects.[31,35-37] The study by Hjerppe and others[31] shows that flexural strength could be improved by surface conditioning with airborne-particle abrasion.

Table 4: *Maximum Profile Valley Depth (*Rv*) of the ZirPlus and Denzir Specimens (μm)*[a]

Specimen	ZirPlus (Mean±SD)	Denzir (Mean±SD)
1.32 mm, as delivered	1.5 ± 0.5 A, E	1.0 ± 0.8 C
1.32 mm, heat treated	1.4 ± 0.5 A, B, E	0.5 ± 0.3 I
0.4 mm, as delivered	0.9 ± 0.4 C	0.6 ± 0.3 I, J
0.4 mm, autoclaved	1.5 ± 0.5 A, B, D	1.2 ± 0.5 D, E
0.4 mm, heat treated	1.3 ± 0.8 A, B, D, E	0.7 ± 0.3
0.4 mm, 0.2 MPa	2.4 ± 0.8 F, M	1.5 ± 0.4 A, B, D, E, K
0.4 mm, 0.2 MPa, heat treated	2.6 ± 0.7 F	1.5 ± 0.3 A, B, D, E, K
0.4 mm, 0.6 MPa	4.5 ± 1.1 G	2.2 ± 0.4 L, M
0.4 mm, 0.6 MPa, heat treated	4.5 ± 1.2 G	2.1 ± 0.4 L
0.4 mm, 0.6 MPa, heat treated and autoclaved	5.4 ± 0.9	2.1 ± 0.4 L, M

[a] *Identical letters indicate absence of statistically significant difference (*$p > 0.05$*). No letter indicates statistically significant difference compared with all the other groups in respective table (*$p < 0.05$*). n = 10 in each group (Mann-Whitney U-tests).*

Table 5: *Maximum Height of the Profile (*Rz*) of the ZirPlus and Denzir Specimens (μm)*[a]

Specimen	ZirPlus (Mean±SD)	Denzir (Mean±SD)
1.32 mm, as delivered	2.5 ± 0.7 A	1.4 ± 0.4
1.32 mm, heat treated	2.5 ± 1.0 A, B	1.0 ± 0.5 F
0.4 mm, as delivered	1.8 ± 0.7	1.0 ± 0.5 F
0.4 mm, autoclaved	2.8 ± 0.9 A, B, C	2.2 ± 0.9 A, B
0.4 mm, heat treated	2.6 ± 1.5 A, B, C	1.2 ± 0.5 F
0.4 mm, 0.2 MPa	4.6 ± 1.4 D	3.1 ± 0.8 C
0.4 mm, 0.2 MPa , heat treated	4.5 ± 1.1 D	3.1 ± 0.7 C
0.4 mm, 0.6 MPa	8.6 ± 1.9 E	4.4 ± 0.9 D, G
0.4 mm, 0.6 MPa, heat treated	8.2 ± 2.1 E	4.2 ± 0.8 D, G, H
0.4 mm, 0.6 MPa, heat treated and autoclaved	9.5 ± 1.4	4.1 ± 0.7 D, H

[a] *Identical letters indicate absence of statistically significant difference (*$p>0.05$*). No letter indicates statistically significant difference compared with all the other groups in respective table (*$p<0.05$*). n = 10 in each group (Mann-Whitney U-tests).*

The studies by Garcia Fonseca and others[34] and Özcan and others[36] demonstrate that the size and type of particles could affect the strength of Y-TZP ceramic and reduce it; these findings provide one possible explanation for the effect of 0.6 MPa sandblasting on the ZirPlus discs. Manufacturing and adjustments involving grinding and/or polishing of zirconia-based restorations could influence the BFS of Y-TZP ceramics and should therefore be carried out carefully.

Effects of Heat Treatment and Accelerated Aging

Heat treatment did not significantly affect the BFS of the 1.3-mm specimens but significantly reduced the BFS of the 0.4-mm discs (Figure 2; Table 1). For the 0.2 MPa sandblasted discs, only the ZirPlus specimens were affected by the heat treatment, whereas heat treatment of the 0.6 MPa sandblasted discs significantly reduced the BFS of both the ZirPlus and the Denzir specimens (Figure 2; Table 2). Another interesting result was that autoclaving did not affect the BFS after heat treatment of the 0.6 MPa sandblasted 0.4-mm ZirPlus and Denzir specimens, whereas the BFS of the as-delivered 0.4-mm specimens was reduced after autoclaving (Figure 2; Tables 1 and 2). These effects on the BFS of autoclave treatment of the ZirPlus and Denzir discs in the current study were similar to the findings reported in the previous study of Mg-PSZ specimens.[2] In that study, it was shown that autoclaving of the as-delivered Mg-PSZ specimens reduced their strength, whereas it had no influence on the BFS of heat-treated 0.6 MPa sandblasted specimens.

The fact that the heat-treated discs were not affected by subsequent autoclaving is of interest since accelerated aging of ceramics by autoclaving at 134°C for five hours has been recommended in ISO 13356; 2015[38] as a method for studying aging of, for example, ceramic femoral heads. In the current study, the specimens were autoclaved at 134°C and 0.2 MPa for 10 hours, which, according to Chevalier and others,[39] corresponds to ~36-48 years *in vivo*. In the previous study by Lucas and others[24] of two brands of Y-TZP, autoclaving for five hours and 0.2 MPa did not influence the modulus of elasticity. It has also been reported in previous studies of zirconia[5,24] that the grain size could influence the t → m transformation[23] and that grains below a certain size seem not to transform t → m.[5] However, it should be noted that in a meta-analysis by Pereira and others[39] of low-temperature degradation of Y-TZP ceramic, it was stated that autoclave aging could reduce the mechanical properties of Y-TZP and that aging time, pressure, and temperature affect the outcome. Another meta-analysis[40] reported that airborne-particle abrasion could both increase and reduce the strength and affect the phase transformation in Y-TZP and that after artificial aging in an autoclave for more than 12 hours, there was less monoclinic content in the sandblasted specimens than in the control. In the present study, both autoclaving and heat treatment could reduce the BFS, and it is of great interest to examine how these methods affect the phase transformation of the studied Y-TZP materials, and further studies are needed to clarify the transformation process.

Surface Roughness and the Pearson Correlation Test

The *Ra*, *Rv*, and *Rz* values of the 0.4-mm ZirPlus and 0.4-mm Denzir specimens in the current study were significantly increased after autoclaving compared to the as-delivered discs (Tables 3 through 5). This is in

agreement with the results in a previous study by Lucas and others[24] addressing the artificial aging of zirconia in autoclaving where a slight increase in surface roughness was seen after aging in an autoclave for five hours. In spite of this, the Pearson correlation test in the present study showed no correlation ($p<0.05$) between the BFS and the surface roughness, except for the heat-treated 0.4-mm Denzir discs and the heat-treated and autoclaved 0.4-mm ZirPlus discs sandblasted with 0.6 MPa. Similar findings were reported in the study by Ramos and others,[26] in which it was stated that no correlation was seen between surface roughness and the BFS of Y-TZP ceramic. On the other hand, in the study by Hjerppe and others,[31] a statistically significant correlation was seen between surface roughness and the strength values in a three-point bend test of zirconia, indicating that the surface roughness could influence the fracture behavior of dental zirconia.

CONCLUSIONS

Based on the findings in the present study, several factors could significantly influence the BFS of the Y-TZP–based ceramics studied. In particular, the thickness of Y-TZP–based ceramics seems to have a major impact on the BFS of the material. In addition, surface treatments, such as sandblasting and heat treatment, have an impact on the BFS in the Y-TZP ceramics tested. Since heat treatment and sandblasting are methods that are commonly used for manufacturing this type of dental ceramic restoration, the surface treatment of Y-TZP ceramics should be carried out with care to avoid unexpected negative effects on the material.

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Conflict of Interest

Dr Anders Sundh has previously been employed (part-time) as manager of research and development at Cad.esthetics AB, Skellefteå, Sweden.

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Bonding Interaction and Shrinkage Stress of Low-viscosity Bulk Fill Resin Composites With High-viscosity Bulk Fill or Conventional Resin Composites

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Clinical Relevance

The use of high-viscosity bulk fill associated with low-viscosity bulk fill resin composites presents an adequate bonding interaction and results in better margin stability due to lower shrinkage stress at the occlusal enamel margin.

SUMMARY

Objective: To analyze the shrinkage stress, bonding interaction, and failure modes between different low-viscosity bulk fill resin composites and conventional resin composites produced by the same manufacturer or a high-viscosity bulk fill resin composite used to restore the occlusal layer in posterior teeth.

Methods & Materials: Three low-viscosity bulk fill resin composites were associated with the conventional resin composites made by the

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same manufacturers or with a high-viscosity bulk fill resin composite, resulting in six groups (n=10). The bonding interaction between resin composites was tested by assessing the microshear bond strength (μSBS). The samples were thermocycled and were tested with 1-mm/min crosshead speed, and the failure mode was evaluated. The post-gel shrinkage (Shr) of all the resin composites was measured using a strain gauge (n=10). The modulus of elasticity (E) and the hardness (KHN) were measured using the Knoop hardness test. Two-dimensional finite element models were created for analyzing the stress caused by shrinkage and contact loading. The μSBS, Shr, E, and KHN data were analyzed using the Student *t*-test and one-way analysis of variance. The failure mode data were subjected to chi-square analysis (α=0.05). The stress distribution was analyzed qualitatively.

Results: No significant difference was verified for μSBS between low-viscosity bulk fill resin composites and conventional or high-viscosity bulk fill composites in terms of restoring the occlusal layer (p=0.349). Cohesive failure of the low-viscosity bulk fill resin composites was the most frequent failure mode. The Shr, E, and KHN varied between low-viscosity and high-viscosity resin composites. The use of high-viscosity bulk fill resin composites on the occlusal layer reduced the stress at the enamel interface on the occlusal surface.

Conclusions: The use of high-viscosity bulk fill resin composites as an occlusal layer for low-viscosity bulk fill resin composites to restore the posterior teeth can be a viable alternative, as it shows a similar bonding interaction to conventional resin composites as well as lower shrinkage stress at the enamel margin.

INTRODUCTION

Resin composites are widely used in posterior restorations with optimum longevity.[1-7] To simplify and streamline the restorative process, bulk fill resin composites were created to reduce the clinical steps and consequently the length of chair time.[8-10]

Bulk fill resin composites represent an innovative class of resin-based materials. These resin composites are commercialized in high viscosity and are easily sculpted, and they can fill a cavity using 4- to 5-mm increments.[8,11,12] Low-viscosity bulk fill resin composites are usually indicated as a base, filling up to 4.0 mm of the dentin portion of the cavity, and most of the bulk fill composite resin needs a coverage of 2 mm of conventional resin composites, which exhibit better wear strength and tensile strength.[8,13-15] Low-viscosity bulk fill resin composites have shown good adaptation to the cavity walls[9] and lower porosity compared to the conventional resin composite when using an incremental technique.[16-18] The mechanical properties of bulk fill resin composites promote lower polymerization shrinkage stress, better stress distribution, and high fracture resistance.[8] However, this performance is clearly material dependent.[8,19] The chemical structures do not differ substantially from those of conventional resin composites since their base monomers are bisphenol A diglycidylmethacrylate (Bis-GMA), bisphenol A polyethylene glycol diether dimethacrylate (Bis-EMA), triethylene glycol dimethacrylate, urethane dimethacrylate (UDMA), and silanized glass filler particles. However, bulk fill resin composites contain modified UDMA and other modulators of the reaction that possess photoactive groups that control the polymerization kinetics and retard the accumulation of the polymerization shrinkage stress.[19,20]

Combinations of resin composites are becoming more frequent due to the association of the low-viscosity bulk fill with the conventional resin composite.[8,13,14] The manufacturers recommend the use of conventional resin composite to cover the low-viscosity bulk fill resin composites at the occlusal surface of the posterior teeth.[21-23] However, clinicians have frequently questioned if the association of the high-viscosity bulk fill and low-viscosity bulk fill resin composites could be a good alternative, aiming to facilitate clinical procedures and to reduce the shrinkage stress.[19] It can be questioned if the chemical interaction between different composites is compromised. No information, to the authors' knowledge, is available regarding the interaction between different low-viscosity bulk fill with conventional or high-viscosity bulk fill resin composites.

Therefore, this study aimed to analyze the shrinkage stress, the bonding interaction, and the failure modes among different low-viscosity bulk fill resin composites with the conventional resin composites produced by the same manufacturer or with one high-viscosity bulk fill resin composite used for restoring the occlusal layer in the posterior teeth. The null hypothesis was that the low-viscosity bulk fill resin composites with the occlusal layer covered using high-viscosity bulk fill or conventional resin composites inserted incrementally would have no effect on bond strength and shrinkage stress.

Table 1: *Resin Composites Used in This Study*

Material	Code	Resin Composite Type	Organic Matrix[a]	Filler[a]	Filler% Wt/Vol[a]	Manufacturer	Lot Number
Filtek Z350XT	Z350	Nanofilled	Bis-GMA, Bis-EMA, UDMA, TEGDMA	Silica and zirconia nanofillers, agglomerated zirconia-silica nanoclusters	78.5/63.3	3M ESPE (St Paul, MN, USA)	N603650
TPH-3	TPH3	Nanohybrid	Bis-EMA, Bis-GMA	Barium glass, barium aluminum silicate, silica	75/57	Dentsply Sirona (Konstanz, Germany)	157722H
Opallis	OPAL	Nanohybrid	Bis-GMA, Bis-EMA, TEGDMA, UDMA	Barium aluminum, silicate, sílica	79/58	FGM (Joinville, Brazil)	300316
Filtek Bulk Fill Posterior	POST	High-viscosity bulk fill	UDMA, DDDMA, EDMAB	Silica, zirconia, YbF3	76.5/58.4	3M ESPE	N690323
Surefil SDR Flow	SDR	Low-viscosity bulk fill	Modified UDMA, dimethacrylate and difunctional diluents	Barium and strontium alumino-fluoro-silicate glasses	68/44	Dentsply Sirona	1508283/150831
OPUS Bulk Fill Flow	OPUS	Low-viscosity bulk fill	TEGDMA, Bis- EMA, UDMA	Silica with urethane dimethacrylate, salinized silica dioxide, salinized barium glass, YbF3	68/—	FGM	060616
Filtek Bulk Fill Flow	FBF	Low-viscosity bulk fill	UDMA, Bis-GMA, EBPADMA, Procrylat resin	Silane-treated ceramic, YbF3	64/42.5	3M ESPE	N768439

[a] *Composition as given by manufacturers.*
Abbreviations: Bis-GMA, bisphenol A diglycidylmethacrylate; Bis-EMA, bisphenol A polyethylene glycol diether dimethacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethyleneglycol dimethacrylate; DDDMA, 1, 12-Dodecanediol dimethacrylate; YbF_3, ytterbium fluoride; EDMAB, tertiary amine ethyl-4-dimethylaminobenzoate; EBPADMA, ethoxylated bisphenol A dimethacrylate.

METHODS AND MATERIALS

The characteristics of the three low-viscosity bulk fill resin composites, three conventional resin composites made by the same manufacturers, and one high-viscosity bulk fill resin composite used in this study are described in Table 1.

Microshear Bond Strength Test

To test the bonding interaction between low-viscosity and the conventional resin composites made by the same manufacturer or the one high-viscosity bulk fill resin composite, a microshear bond test was used. The combinations between resin composites resulted in six groups (n=10):

- FBF/Z350: Filtek Bulk Fill Flow (3M ESPE, St Paul, MN, USA) and Filtek Z350 XT (3M ESPE)
- FBF/POST: Filtek Bulk Fill Flow and Filtek Bulk Fill Posterior (3M ESPE)
- SDR: Surefill SDR (Dentsply Sirona, Konstanz, Germany) and TPH3 Spectrum (Dentsply Sirona)
- SDR: Surefill SDR and Filtek Bulk Fill Posterior
- OPUS: Opus Bulk Fill Flow (FGM, Joinville, Brazil) and Opallis (FGM)
- OPUS: Opus Bulk Fill Flow and Filtek Bulk Fill Posterior

The resin composite samples were embedded into the polystyrene resin cylinders that were 30 mm in height and 20 mm in diameter after flattening and finishing the base and top surfaces using abrasive grit sandpaper of grit sizes 180, 320, and 600 (Norton, Campinas, Brazil). Using a no. 1052 diamond bur (KG Sorensen, Barueri, Brazil), a cylindrical cavity 1 mm thick and 4 mm in diameter was prepared to be filled by the low-viscosity bulk fill resin composites. The low-viscosity resin composites were inserted into the cylindrical cavity and light cured using a broad-spectrum LED light-curing unit (LCU) (VALO, Ultradent, South Jordan, UT, USA) with average irradiant intensity of 1400 mW/cm^2 checked by using a MARC Resin Calibrator (Blue-Light Company, Halifax, NS, Canada). The LCU was rigidly fixed by using a support device (Odeme, Luzema, Brazil) at a distance of approximately 1.0 mm from the cylinder to standardize the distance to the LCU.

To standardize the size of the specimens and to delimit the bonding area, transparent flexible tubes 1.0 mm in diameter and 4.0 mm high were used

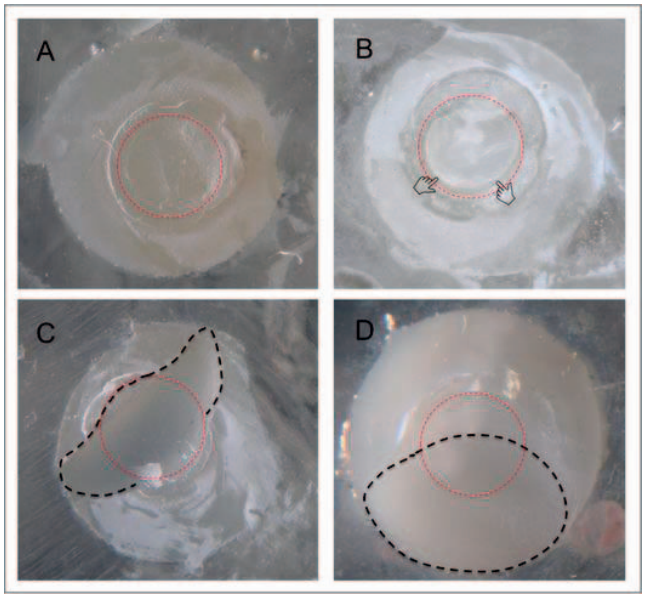


Figure 1. *Failure modes. (A): Adhesive failure. (B): Cohesive failure of occlusal resin composite layer. (C): Cohesive failure of low-viscosity bulk fill resin composite. (D): Mixed failure involving cohesive failure and adhesive failure.*

(Embramac, Campinas, Brazil). The conventional and high-viscosity bulk fill resin composites were inserted into the plastic tube, previously positioned over the center of the low-viscosity bulk fill resin composite samples, and were light cured following the same protocol described above. The plastic tubes were cut longitudinally with a no. 12 scalpel blade (Swann Morton, Rio de Janeiro, Brazil) and were carefully removed by the same operator. The samples were thermally cycled (ER 26000, Erios, São Paulo, Brazil), following a protocol of 6000 cycles between the temperatures of 5°C and 55°C. The specimens were stored at 37.7°C for 24 hours in distilled water before thermal cycling.

The specimens were attached to a specific device fixed to a universal testing machine (Microtensile Machine OM 100, Odeme) so that the resin cylinder's long axis was parallel to the horizontal plane. A 0.3-mm-diameter wire (Morelli, Sorocaba, Brazil) was placed around the resin cylinder over its interface with the surface of the low-viscosity bulk fill composite resin. A 50 N load cell was used to apply an increasing parallel force to the adhesive area at a speed of 1 mm/min until the specimen failure occurred. At the time of fracture, the force was recorded (N) and divided by the area ($A=\pi r^2$, mm) that corresponded to the interface between both resin composites, resulting in microshear bond strength (μSBS) values in MPa.

Failure Mode Analysis

After the μSBS test, the specimens were analyzed using stereomicroscopy at 40× magnification (Mitutoyo, Kawasaki, Japan) to classify the failure mode as follows: type I, adhesive failure between both resin composites; type II, cohesive failure of the covering resin (high-viscosity bulk fill or conventional resin composites); type III, cohesive failure of the low-viscosity composite resin; and type IV, mixed fracture involving part of the low-viscosity bulk fill resin composite and cover composite resin (Figure 1).

Post-Gel Shrinkage

The post-gel shrinkage (Shr) was calculated for all the tested resin composites (n=10) using the strain gauge method.[24] The materials were shaped into semispheres on the top of a biaxial strain gauge (CEA-06-032WT-120, Measurements Group, Raleigh, NC, USA) that measured shrinkage strains in two perpendicular directions. A strain conditioner (ADS05000IP, Lynx Tecnologia Eletrônica, São Paulo, Brazil) converted electrical resistance changes in the strain gauge to voltage changes through a quarter-bridge circuit with an internal reference resistance (120 Ω). The strain values measured along the two axes were averaged since the material properties were homogeneous and isotropic on a macro scale. All the materials were light cured using an LED LCU (VALO, Ultradent) with the light tip held at a 1-mm distance from the surface of the composite. The strain values were collected for five minutes. The maximum shrinkage strain at five minutes was used as the linear post-gel shrinkage input for finite element analysis and could be converted to volumetric percentage by multiplying by 3 and 100%.

Knoop Microhardness (KHN) Measurements

For calculating the modulus of elasticity and the hardness of the tested resin composites, the Knoop hardness test was used.[25] A microhardness tester (FM-700, Future-Tech Corp, Kanagawa, Japan) was used with a diamond indenter to apply a static charge of 100 g (0.98 N) for 10 seconds. For each specimen, the average of five indentations was used on the top surface. The following formula was used: $KHN = 14.229 \times \text{Load (kg)}/(\text{long diagonal in mm})$.[25] In addition to the hardness determination, Knoop indentations were also used to determine the modulus of elasticity.[25,26] The decrease in the length of the indentation diagonals caused by elastic recovery of a material are related to the hardness:modulus ratio (H/E) by the equation

Table 2: *Mechanical Properties Applied for the Dental Structures and Materials*

Structure/ Materials	Modulus of Elasticity (MPa)	Poisson's Ratio	Compressive Strength (MPa)	Diametral Tensile Strength (MPa)	References
Enamel	84,100	0.30	—	—	44
Dentin	18,600	0.30	—	—	45
Pulp	2	0.45	—	—	46
FBF	—	0.24*	229.1	43.8	47
OPUS	—	0.24*	152.5	34.3	47
SDR	—	0.24*	182.3	43.5	8
Z350 XT	—	0.24*	257	47.3	8
OPALLIS	—	0.24*	141	42.1	47
TPH3	—	0.24*	136.2	40.5	47
POST	—	0.24*	169.3	42.4	47

* *Reference 50.*

$$b'/a' = b/a - \propto 1(H/E)$$

where b/a is the ratio of the diagonal dimensions a and b in the fully loaded state, given by a constant 0.140647; b′/a′ is the ratio of the altered dimensions when fully recovered; and $\propto 1 = 0.45$ is a proportionality constant.[26]

Two-Dimensional Modeling of Dynamic Finite Element Molars

Two-dimensional models were created for finite element analysis, simulating a model of a maxillary human first molar in occlusal antagonist contact, from a transverse cone beam tomographic image of a patient with normal occlusion from a bank of dental school images. An occlusal cavity 6 mm in depth was simulated. The coordinates and points of the structures were drawn using processing software (IMAGEJ, public domain, National Institutes of Health, Bethesda, MD, USA) and were imported into a finite element analysis package (Marc & Mentat 2010.2 software, MSC, Santa Ana, CA, USA). Cube spline curves were then created through these coordinates to re-create the contours of the structures for the model.

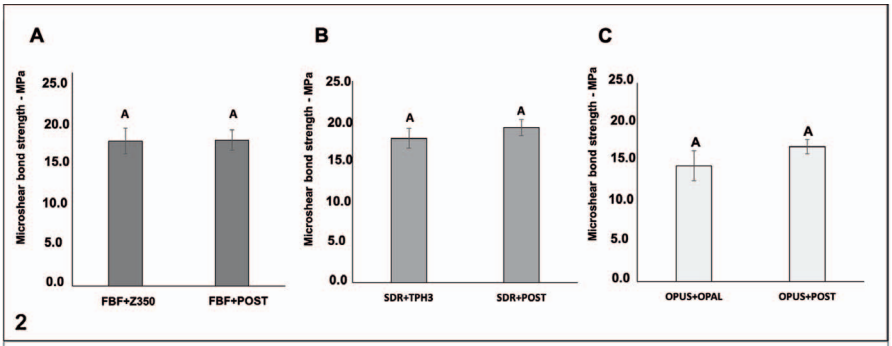
The models were generated following two conditions: 1) cavity restored with 4.0 mm of low-viscosity bulk fill resin composites to replace dentin and covered with 2.0 mm of a high-viscosity bulk fill resin composite in a single increment and 2) molar restored with 4.0 mm of low-viscosity bulk fill resin composites to replace dentin and covered with two increments of 2.0 mm of conventional resin composite. The mesh was created through a manual process that used isoparametric four-node arbitrary quadrilateral deformation elements with reduced integration (one integration point per element), using a no. 115 element type in the software Marc. All the interfaces were attached, considering bonded interfaces. Displacement was limited at the nodes of the base of the maxillary and mandibular bone in the X and Y directions. All the materials were considered linear, isotropic, and homogeneous. The applied mechanical properties are listed in Table 2.[8,27-30] The insertion of the composite resin layers was simulated, and their Shr values were previously calculated. The occlusal contact of 100 N of the mandibular molar with the maxillary molar, followed by a sliding movement, was simulated by using friction contact between the mandibular and maxillary occlusal surfaces (frictional coefficient 0.5). Stress distributions were analyzed using modified von Mises (MVM) stresses, which integrate all the tension components in a value equivalent to stress.

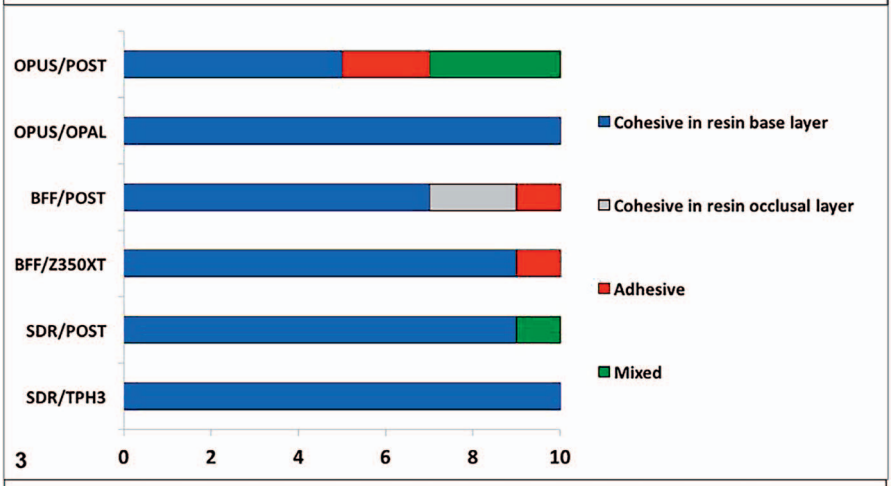
Statistical Analysis

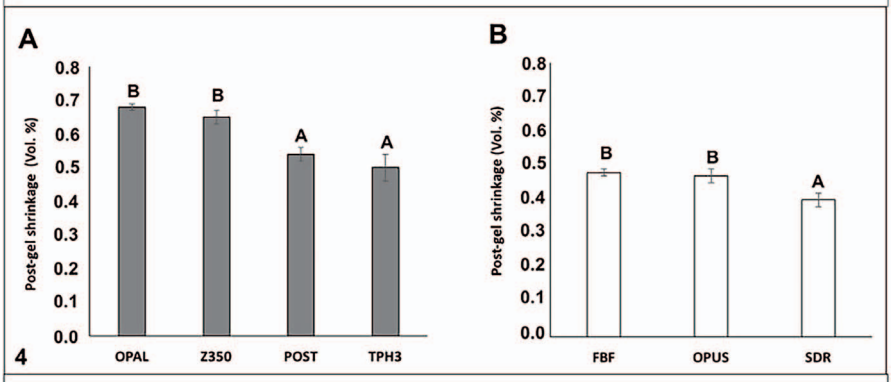
The μSBS, Shr, and KHN data were tested for a normal distribution (Shapiro-Wilk) and for equality of variances (Levene test), followed by parametric statistical tests. The Student *t*-test was performed for the μSBS values. One-way analysis of variance (ANOVA) was performed for the KHN and Shr values. Multiple comparisons were made using the Tukey test. The failure mode data were subjected to chi-square analysis. All the tests employed an $\alpha = 0.05$ significance level, and all the analyses were carried out with the statistical package Sigma Plot version 13.1 (Systat Software Inc, San Jose, CA, USA). Stress distribution was analyzed qualitatively.

RESULTS

The mean μSBS and standard deviation values between the low-viscosity bulk fill and the conven-







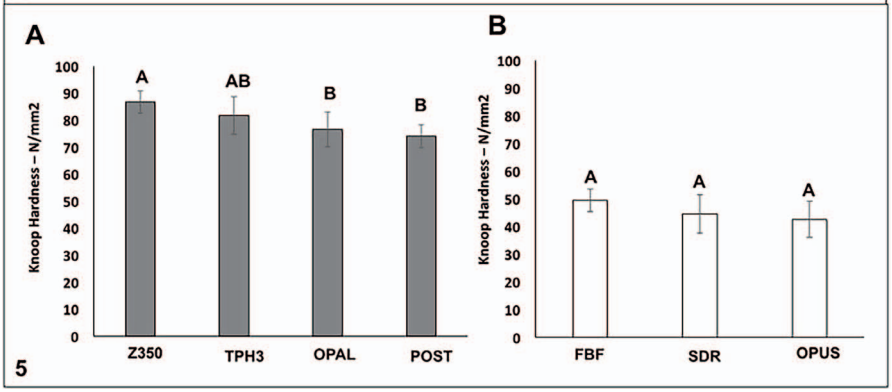


Figure 2. *Mean and standard deviation microshear bond strength (μSBS) for the two experimental conditions tested for each low-viscosity bulk fill resin composites. (A): FBF. (B): SDR. (C): OPUS covered by conventional resin composites (Z350, TPH3, and OPAL, respectively) or high-viscosity composite resin (POST). Different letters indicate significant differences tested by the Student* t*-test (*$p<0.05$*).*

Figure 3. *Distribution of failure modes for the different experimental conditions.*

Figure 4. *Mean and standard deviation of post-gel shrinkage. (A): Conventional resin composites and high-viscosity bulk fill resin composite. (B): Low-viscosity bulk fill resin composites. Different letters demonstrate significant differences among the tested resin composites for each group (low and high viscosity).*

Figure 5. *Means and standard deviations of the microhardness Knoop (KHN) of the composites. (A): Conventional and high-viscosity bulk fill resin composites. (B): Low-viscosity bulk fill resin composites. Different letters demonstrate significant differences among the tested resin composites for each group (low and high viscosity).*

tional resin composites made by the same manufacturer as well as the Filtek Bulk Fill Posterior resin composite are shown in Figure 2. The Student *t*-test showed no significant difference for the bonding strength between low-viscosity bulk fill resin composites and the conventional resin composites produced by the same manufacturer or the high-viscosity bulk fill resin composite. The failure mode distribu-

tions for the tested samples are shown in Figure 3. No significant difference was found for the failure mode among the groups ($p=0.134$). Cohesive failures of low-viscosity bulk fill resin composites were the prevalent failure mode observed for all the groups.

The mean Shr and standard deviation values for all the resin composites are shown in Figure 4. One-way ANOVA showed that the SDR had lower Shr values than the FBF and OPUS. POST and TPH3 had similar Shr values and lower values than the FZ350 and OPAL resin composites.

The mean KHN and standard deviation values for all the resin composites are shown in Figure 5. One-way ANOVA showed that Z350 had higher KHN values than OPAL and POST as well as similar values to that of TPH3 ($p<0.01$). POST, TPH3, and OPAL had similar KHN values ($p=0.231$). Comparing the low-viscosity bulk fill resin composites, one-way ANOVA showed no significant difference among KHN means ($p=0.341$).

The mean E and standard deviation values for all the resin composites are shown in Figure 6. Comparing the low-viscosity bulk fill resin composites, one-way ANOVA showed that SDR showed higher E values than FBF and OPUS ($p<0.01$). Comparing the resin composites used for the occlusal layer, TPH3 and Z350 had higher E values than OPAL and POST.

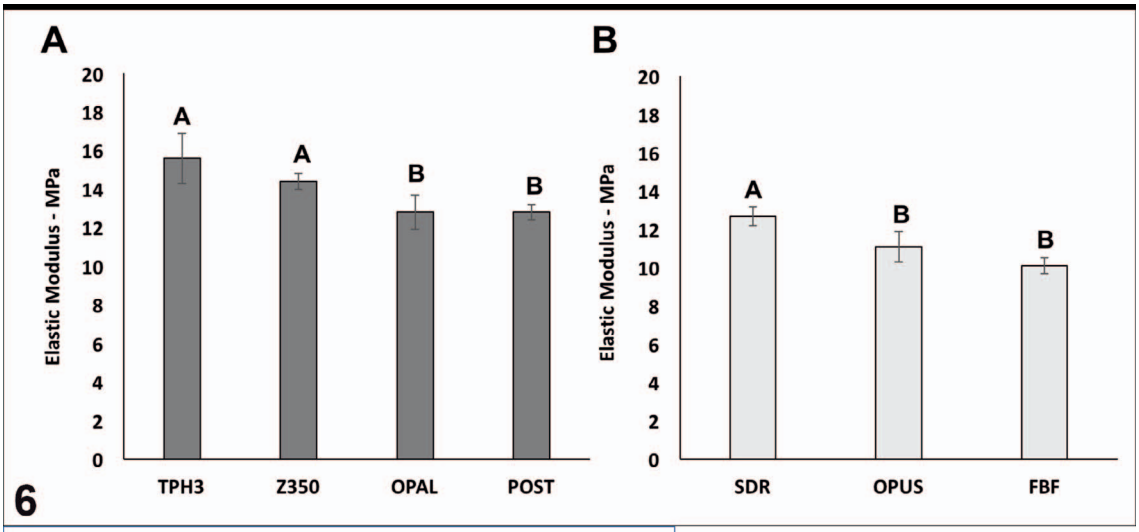
The stress distributions during restoration and at 100 N occlusal loading (modified von Misses stress) are shown in Figure 6. The use of the high-viscosity bulk fill (POST) on the occlusal layer resulted in lower shrinkage stress concentration at the enamel interface than when conventional resin composites inserted incrementally were used regardless of the tested resin composite combinations. OPAL had a higher critical von Mises stress at the enamel interface and at the walls of the restoration, mainly on the lingual cusp, than all the other conventional resin composites (Figure 6).

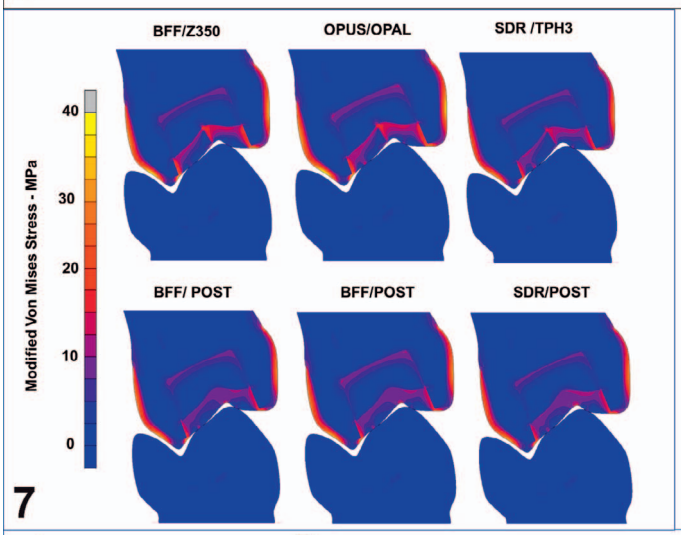
The stress distribution during restoration and occlusal function in the enamel, dentin, low-viscosity composite resin, and occlusal composite resin layers is shown in Figure 7. The maximum peak stress was observed on the enamel structure during the restoration procedure and during the antagonist tooth contact with the enamel/composite resin interface. The stress level of the enamel and dentin structures was lower when POST was used to restore the occlusal layer. The MVM stress was recorded at 18 points along the composite resin/tooth structure interface, as shown in Figure 8. The use of the high-viscosity bulk fill resin composite for restoring the occlusal layer reduced the stress at the margin of the restoration.

DISCUSSION

The bond strengths between low-viscosity bulk fill resin composites and high-viscosity bulk fill or a conventional resin composite were similar; therefore, the first null hypothesis was not rejected. However, the shrinkage stress generated at the enamel/restoration occlusal margins was lower when using the high-viscosity bulk fill resin composite for covering the low-viscosity bulk fill resin composites; therefore, the second null hypothesis was rejected. The combination of different low-viscosity bulk fill resin composites with high-viscosity bulk fill or conventional resin composites used to cover the occlusal surface had no influence on the bonding interaction among these materials. Although the bulk fill resin composites have the chemical modifications in their formulations to facilitate deep polymerization, the monomer interaction with the bulk fill low-viscosity resin was similar when compared with resin composites produced by the same manufacturer. The tested materials presented similar variations of the monomers and modulators in their compositions. However, the UDMA and Bis-GMA or Bis-EMA monomers were present in most resin composites, facilitating the interaction between different low-viscosity resin composites with the occlusal-layer resin composites.[31-33] The present study confirmed that the interaction with conventional and bulk fill resin composites performed similarly to the interaction between high-viscosity bulk fill resin composite.

The microshear method allows small areas to be effectively tested; however, during the test, stress was most concentrated at the base substrate,[34,35] in this study represented by the low-viscosity composite resin. These facts induced premature failure of the specimens.[35-37] The lower filler content present in low-viscosity bulk fill resin composites is reflected in their mechanical properties, resulting in decreased modulus of elasticity and hardness values.[10,11,37] These aspects may explain the high frequencies of the observed failure mode involving low-viscosity bulk fill resin composites. The presence of a high frequency of cohesive failure can mask the real difference on the bonding interaction between different resin composites. This study found no difference for the failure mode for all the tested combinations, probably because of the large similar-





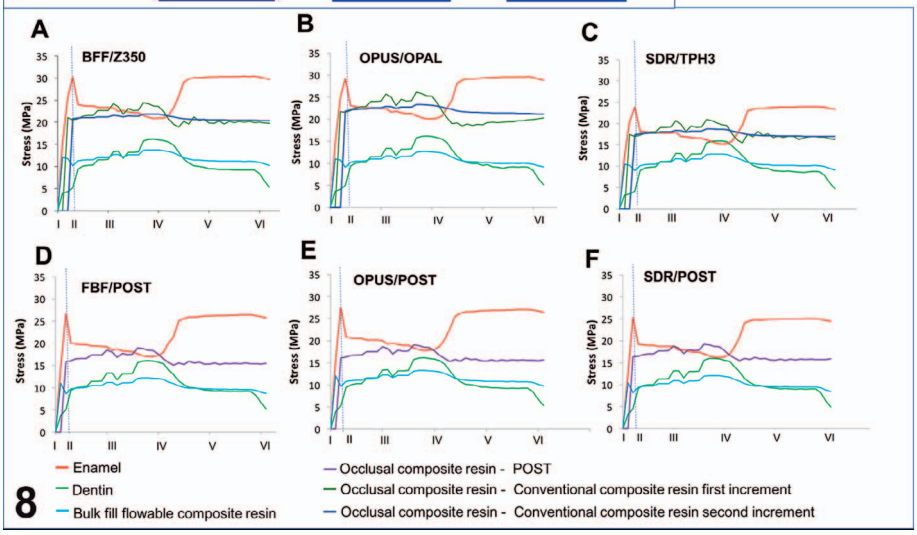


Figure 6. *Mean and standard deviation of the modulus of elasticity. (A): Conventional and high-viscosity bulk fill resin composites. (B): Low-viscosity bulk fill resin composites. Different letters demonstrate significant differences among the tested resin composites for each group (low and high viscosity).*

Figure 7. *Modified von Mises stress distribution generated by shrinkage and occlusal loading for different resin composite combinations tested at the maximum intercuspation of the molar teeth.*

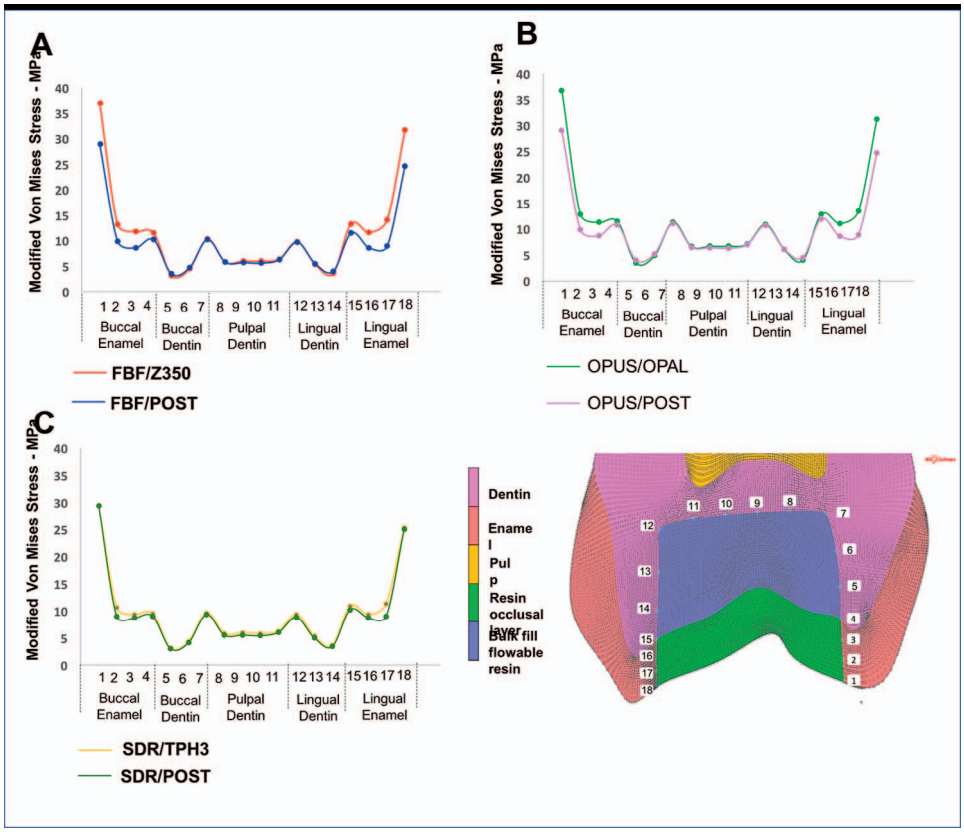


Figure 9. *The values of modified von Mises stress generated at the tooth structure/resin composite interface caused by shrinkage and occlusal loading for different resin composite combinations.*

ity on the filler content of the low-viscosity bulk fill resin composites.

During the polymerization of the resin composites, the modulus of elasticity develops when the material becomes rigid and its original capability for plastic flow as a paste decreases.[39,40] Shrinkage stress is generated when the composite material becomes solid enough to transfer stresses that can no longer be relieved by flow.[19,25] The modulus of elasticity tends to correlate with the hardness of the material as well as with the filling content.[38-40,41] The post-gel shrinkage and the modulus of elasticity of the material can influence the magnitude of the stress generated during polymerization.[19,25] Manufacturers incorporate inorganic fillers in resin composites with the aim of improving the mechanical properties of resin composites.[43] Bulk fill resin composites have a chemical composition similar to that of conventional resin composites in both the organic and the inorganic matrix.[44] However, these materials have modulators for the reaction that can explain the lower post-gel shrinkage and modulus of elasticity values compared with the conventional resin composites. Low-viscosity bulk fill resin composites exhibited the lowest post-gel shrinkage and modulus of elasticity because of decreased filler content and a higher capacity for intrinsic deformation, releasing the stress generated during polymerization shrinkage.[8] The shrinkage stress calculated at final restoration was the combination of the performance of both resin composites used in each group. The SDR/TPH3 or SDR/POST were the groups with the lowest shrinkage stress, probably because of the combination between the lowest shrinkage of SDR among low-viscosity bulk fill resin composites and lower Shr values of TPH3 Spectrum and Filtek Bulk Fill Posterior.

Considering that shrinkage stresses are concentrated at the occlusal margin,[42,45] the combination of shrinkage stresses and occlusal loading may be a determining factor in the mechanical performance of a restorative complex.[45] The stress concentrated at the enamel/occlusal margin may determine the marginal fracture or the marginal debonding.[19] If

←
Figure 8. *The 10% highest values of modified von Mises stress generated on the enamel, dentin, the low-viscosity bulk fill resin composite, and the occlusal resin composite, caused by shrinkage and occlusal loading for different composite resin combinations. (A): FBF/Z350. (B): OPUS/OPAL. (C): SDR/TPH3. (D): FBF/POST. €: OPUS/POST, SDR/POST.*

the occlusal contact is located at the margin of a composite restoration, the increased stress concentration may increase the risk of marginal fracture. A retrospective clinical study showed that the fracture of restorations was the main reason for failure in "occlusal-stress-risk" patients.[45] Even if marginal deterioration is too small to be perceived clinically, it may increase the retention of pigments, increasing marginal discoloration. As marginal staining can be confused with marginal caries, such restorations may be replaced prematurely.[46]

It has frequently been indicated that low-viscosity bulk fill resin composites should be covered with a 1.5- to 2.0-mm layer with conventional resin composite.[8,11,12] The hardness of the material is one of the factors for the selection of the material when restoring posterior teeth[8,11,12] as well as the wear resistance.[37,47] It has been reported that bulk fill resin composites have adequate polymerization and hardness of the material not only on the surface of the resin composite but also in the depth of the restoration,[8,42] which was demonstrated in the present study, where the high-viscosity bulk fill composites had KHN values similar to those of conventional resin composites. However, the low-viscosity bulk fill resin composites had lower KHN values, which can be attributed to the low modulus of elasticity and filler content in its chemical composition. The results of the present study confirmed the difference in KHN hardness and that lower values of the low-viscosity resin composites reflect the necessity for the covering, preventing wear and bulk fracture.[47-49] Additionally, this study demonstrated that the use of high-viscosity bulk fill could be an adequate alternative for restoring the occlusal surface of posterior restorations.

This study has the limitation of testing only one high-viscosity resin composite. The shrinkage stress impacts are observed during the early stages regarding bond strength; hence, bond strength data gathered before the thermal cycling could add important information. Unless all factors can be modeled, the results of a finite element analysis should still be carefully interpreted within the clinical context.[18,24] Observing these clinical procedures with an understanding of the balance between mechanical properties offered by various composites may improve the clinical performance of posterior resin composite restorations. The use of low-viscosity bulk fill resin composites results in better adaptation and lower bubble formation.[17] When high-viscosity bulk fill material is used for restoring the occlusal surface, this tends to be a good option for posterior restorations.

CONCLUSIONS

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

1) There was no difference in bond strength between conventional or high-viscosity bulk fill resin composite with low-viscosity bulk fill resin composites. The cohesive failure mode was the most frequent mode in all the groups.
2) High-viscosity bulk fill resin composites had lower Shr values than conventional resin composites.
3) The modulus of elasticity varied substantially among the low-viscosity bulk fill resin composites.
4) High-viscosity bulk fill composite resin (Filtek Posterior) has similar KHN values to the conventional resin composites, which are normally recommended to fill the occlusal surface of low-viscosity bulk fill resin composites.
5) The use of high-viscosity bulk fill resin composites on the occlusal layer resulted in a decreased stress concentration at the enamel interface compared to the conventional resin composites inserted incrementally.

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Regulatory Statement

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

Conflict of Interest

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

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In Vitro Evaluation of Surface Properties and Wear Resistance of Conventional and Bulk-fill Resin-based Composites After Brushing With a Dentifrice

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Clinical Relevance

Gloss retention, surface smoothness, and wear resistance are important factors when choosing resin-based composites.

SUMMARY

Objectives: This study evaluated the effect of toothbrushing with a dentifrice on gloss, roughness profile, surface roughness, and wear of conventional and bulk-fill resin-based composites.

Methods and Materials: Gloss and surface roughness of resin-based composites (RBCs; Admira Fusion X-tra, Aura Bulk Fill, Filtek Bulk Fill Flowable, Filtek Bulk Fill Posterior Restorative, Filtek Supreme Ultra, Herculite Ultra, Mosaic Enamel, SDR flow+, Sonic Fill 2, Tetric EvoFlow Bulk Fill and Tetric EvoCeram

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Bulk Fill) were analyzed before and after brushing; the roughness profile and wear were also determined after toothbrushing. Representative three-dimensional images of the surface loss and images comparing the unbrushed and brushed surfaces were also compared. Analysis of variance and Tukey post hoc tests were applied (α=0.05) to the gloss, surface roughness, roughness profile, and surface loss data. Pearson's correlation test was used to determine the correlation between gloss and surface roughness, surface loss and percentage of gloss decrease after brushing, and surface loss and surface roughness after brushing.

Results: For all RBCs tested after 20,000 brushing cycles, the gloss was reduced and the surface roughness increased ($p<0.05$). However, the roughness profile and the amount of surface loss were dependent on the RBC brand. Admira Fusion X-tra, Aura, Tetric EvoCeram Bulk Fill, and Tetric EvoFlow Bulk Fill showed the deepest areas of wear ($p<0.05$). A significant negative correlation was found between gloss and surface roughness, and a weak correlation was found between the decrease in gloss and the extent of surface loss, and any increase in surface roughness and the surface loss.

Conclusions: Toothbrushing with a dentifrice reduced the gloss, increased the surface roughness, and caused loss at the surface of all the RBCs tested. Considering all the properties tested, Mosaic Enamel exhibited excellent gloss retention and a low roughness profile and wear, while Admira Fusion X-tra exhibited the greatest decrease in gloss, the highest roughness profile, and the most wear.

INTRODUCTION

Resin-based composites (RBCs) have evolved significantly in terms of their use of filler content, resin matrix, and initiator systems.[1-3] These efforts have focused on improving the strength and wear resistance and decreasing the polymerization shrinkage stress of the RBCs. The features should enhance the clinical longevity of restorations, decrease the complexity of the restorative procedure, and decrease chairside time.[2,4,5]

Incremental filling and light curing have been used successfully for many years and are intended to both improve the margin adaptation and reduce the shrinkage stress after photocuring the RBC.[6,7] The introduction of bulk-fill RBCs has meant that instead of using the traditional 2-mm increment composite filling and curing technique, a 4- or 5-mm increment of bulk-filled RBC can now be successfully light cured.[4,8]

Bulk-fill composites have been classified according to their viscosity.[4] Low-viscosity bulk-fill composites (flowable composites) generally have a lower filler content and are best used as a base or for small restorations. High-viscosity bulk-fill RBCs generally have higher filler content and can be used to cover the softer flowable RBCs or they can be used to fill entire restorations because they have better wear resistance and improved mechanical properties compared with flowable RBCs.[4,9] Differences in filler content are also found within conventional RBCs, mostly with respect to particle size and shape. Smaller particle sizes will often produce RBCs that have greater surface gloss retention and improved wear resistance.[10,11] Besides the filler size and shape, the hardness of these fillers, the strength of the bond between the inorganic content and polymer matrix, and the light curing of the RBC can also affect the wear resistance.[12] Consequently, surface properties such as gloss, roughness, and wear resistance will vary among different RBCs. The surface gloss and smoothness of the composite surface are factors involved in the esthetic appearance of the restoration; glossier restorations may provide a better match to the surrounding tooth structure.[13] In addition, it has been reported that there is a significant relationship between the gloss and the surface roughness of RBCs.[13,14]

One of the main reasons for replacing an RBC restoration is the recurrence of tooth decay.[5] Surface roughness plays a crucial role in the amount of bacterial adhesion, biofilm accumulation, and surface staining.[15] Microbial colonization begins in the surface irregularities in which the bacteria can grow protected from the hydrodynamic shear forces and in which cleaning is also more difficult. This results in increased bacterial growth[16,17] and an increase in the risk of caries or periodontal disease.[18]

Therefore, it is clinically relevant to investigate the effect of toothbrushing with a dentifrice on the surface gloss, surface roughness, roughness profile, and the wear resistance of different commercially available RBCs. The null hypotheses of this study were as follows:

1. Toothbrushing would not affect the gloss retention of RBC specimens.

2. Toothbrushing would not affect the surface roughness of RBC specimens.
3. The roughness profile after brushing would not vary among different types of RBCs.
4. Surface wear after brushing would not vary among different types of RBCs.
5. There would be no correlation between the investigated properties.

METHODS AND MATERIALS

RBCs

To measure the effect of toothbrushing with a dentifrice on the change in gloss, surface roughness, roughness profile, and wear, a wide range of commercially available RBCs was chosen for this *in vitro* study. Table 1 describes the RBCs that were tested and their classifications. Sonic Fill 2 was delivered using two different sonication settings on the handpiece: 1 (slowest delivery) and 5 (fastest delivery).

Specimen Preparation and Brushing Cycling

A multiple-peak light-emitting diode light-curing unit (LCU; Valo Grand, Ultradent Products Inc, South Jordan, UT, USA; serial No. MFG3277-5) was used on the standard setting for 20 seconds to light cure all the RBCs. On this setting, the LCU delivered a radiant exitance of 953 mW/cm^2 and an emission spectrum from 380 nm to 490 nm, with three emission peaks (at 396, 447, and 466 nm). The output from the LCU was measured using a 6-inch integrating sphere (Labsphere, North Sutton, NH, USA) that was attached to a fiber-optic spectrometer (USB-4000, Ocean Optics, Dunedin, IL, USA). Five disks of each RBC were made on top of a Mylar strip in metal molds that were 2-mm thick and 12.7-mm in diameter. After the RBC was placed into the mold, it was covered with a Mylar strip and pressed flat with a glass plate, to obtain a flat and smooth surface. Then, the glass plate was removed, and the specimens were light cured, using the LCU that was held 2 mm away from the RBC surface. The Mylar strips were removed from the top and bottom surface of the RBCs after light curing, and the specimens were stored in the dark at 37°C. After 24 hours of storage, the initial gloss and roughness measurements were made. Adhesive tape (Scotch Commercial Vinyl Electrical Tape 700, 3M Electrical Markets Division, Austin, TX, USA) was then applied to only half of the top surface of the RBC disk to protect this area of RBC from brushing. This half of the specimen served as the control area (unbrushed RBC) for the wear measurements. The other half of each composite disk was brushed for 25,000 reciprocal strokes using an Ultradent brushing unit,[19] with a brushing speed of 2.5 cm/s. The toothbrushes moved horizontally, back and forth, while the sample holder rotated. It is considered that between 10,000 and 14,600 back-and-forth brushing cycles correspond to ~1 year of *in vivo* toothbrushing.[19] In this study, the 25,000 reciprocal strokes may be considered to be approximately 2 years of *in vivo* toothbrushing. Soft toothbrushes (Colgate 360° Soft, Colgate Oral Pharmaceuticals Inc, Toronto, Ontario, Canada) and a toothpaste (Colgate Optic White, Colgate Palmolive Canada Inc, lot No. 6150MX1134, RDA: 101) solution (50 g of toothpaste to 80 mL of deionized water) were used to brush the RBC disks with a 180-g force. This is a typical load used in other brushing studies and is similar to the 150-g load that is used in the ISO standard.[19-21] After brushing, the adhesive tape was removed, and the RBC disks were thoroughly washed and air dried.

Gloss Retention

The surface gloss (gloss units [GU]) was measured with the Novo-Curve glossmeter (Rhopoint Instruments Ltd, Hastings, Sussex, UK) at a 60° angle of illumination. The device has a 4.5-mm aperture and was calibrated (93.3 GU) with a plate provided by the manufacturer before the measurements. The first measurements (unbrushed) were made after 24 hours of storage and before the adhesive tape was applied to avoid any interference of the black adhesive tape on the gloss measurement. Similar to other studies, no finishing or polishing was done to compare the gloss achieved by each RBC against the Mylar surface.[19] After brushing, the brushed side could be visually detected by the loss of gloss. The brushed side of the disks was positioned over the aperture of the glossmeter. Three gloss measurements were made on each specimen before and after brushing, and an average of these three measurements was used. The results for each RBC were expressed in GU and analyzed using a two-way repeated-measures analysis of variance (ANOVA) that was followed by Tukey post hoc multiple-comparison tests (α=0.05).

Surface Roughness and Roughness Profile

After the gloss measurements, the surface roughness and roughness profile were measured using a confocal microscope (LEXT 3D Measuring Laser Microscope OLS4000, Olympus Corp, Tokyo, Japan) and OLS4000 software (Olympus Corp). A nonde-

Table 1: *RBC Manufacturer, Classification, Lot Number, and Shade*

RBC	Manufacturer (Address)	Type	Lot No.	Shade	Filler Loading, Type and Size
Admira Fusion X-tra	Voco GmbH (Cuxhaven, Germany)	Bulk-fill nanohybrid Ormocer	1604142	U	84.0% weight per weight. Silicon dioxide nanofillers (~20-50 nm) and silicon oxide–based hybrid fillers (~1 μm).
Aura Bulk Fill	SDI Limited (Bayswater, Victoria, Australia)	Full-body bulk-fill nanohybrid	160340	BKF	81 % by weight. Amorphous SiO_2, barium aluminosilicate glass, pre polimerized filler. Particle size not stated.[a]
Filtek Bulk Fill Flowable	3M ESPE (St Paul, MN, USA)	Flowable bulk-fill microhybrid		A2	64.5% by weight (42.5% by volume). Zirconia/silica particle size that ranges from 0.01-3.5 μ. The average particle size is 0.6 μ. The ytterbium trifluoride has a particle size range of 0.1-5.0 μ.
Filtek Bulk Fill Posterior	3M ESPE (St Paul, MN, USA)	Full-body bulk-fill nanofilled	N771662	A2	76.5% by weight (58.4% by volume). Non agglomerated/non aggregated 20-nm silica filler, a non agglomerated/non aggregated 4- to 11-nm zirconia filler, an aggregated zirconia/silica cluster filler (composed of 20-nm silica and 4- to 11-nm zirconia particles), and a ytterbium trifluoride filler consisting of agglomerate 100-nm particles.
Filtek Supreme Ultra	3M ESPE (St Paul, MN, USA)	Conventional nanofilled	N788069	A2 Body	78.5% by weight and 63.3% by volume. A combination of silane-treated nanoclusters and individual silane-treated nanosilica and nanozirconia. The non agglomerated and non aggregated silica filler is ~20 nm. The non agglomerated/ non aggregated zirconia filler is ~4-11 nm in size.
Herculite Ultra	Kerr Corporation (Orange, CA, USA)	Conventional nanohybrid	6063375	A2 Enamel	78% by weight. Barium glass filler of 0.4 μm average size and silica nanofiller (20-50 nm).
Mosaic	Ultradent Products Inc. (South Jordan, UT, USA)	Conventional nanohybrid	BDZ19	Enamel	68% by volume for the dentin shades, and 56% for the enamel shades. Zirconia-silica glass ceramic and 20 nm silica.
SDR flow+	Dentsply Sirona (Milford, DE, USA)	Flowable bulk-fill nanohybrid	160910	A2	70.5% by weight (47.3% by volume). Barium-alumino-fluoro-borosilicate glass; strontium alumino-fluoro-silicate glass; surface-treated fumed silica; YbF3 inorganic particle size ranging from 20 nm to 10 μm.
Sonic Fill 2 (settings 1 and 5)	Kerr Corporation (Orange, CA, USA)	Bulk-fill nanohybrid		A2	81.35% weight per weight. Silica, barium glass, YbF3, mixed oxides. Particle size not stated.
Tetric EvoCeram Bulk Fill	Ivoclar Vivadent (Schaan, Liechtenstein)	Full-body bulk-fill nanohybrid	V23428	IVA	76%-77% by weight (53%-54% by volume). Barium aluminum silicate glass with two different mean particle sizes, an "Isofiller," ytterbium fluoride, and spherical mixed oxide. The standard filler content is ~61% (vol.) plus 17% Isofillers cured dimethacrylates, glass filler, and ytterbium fluoride. Particle sizes between 40 nm and 3 μm. The prepolymers include inorganic and organic products and are ~25 μm in size.
Tetric EvoFlow Bulk Fill	Ivoclar Vivadent (Schaan, Liechtenstein)	Flowable bulk-fill microhybrid	V28277	IVA	68.2% by weight (46.4 by volume). Barium glass, ytterbium trifluoride, and copolymers (71 wt%). The particle size of the inorganic fillers ranges between 0.1 μm and 30 μm, with a mean particle size of 5 μm.

Data provided by the manufactures.
[a] *According to Karacolak and others (2017).*

structive three-dimensional analysis from a predetermined area of 6.76 mm^2 (2.6 × 2.6 mm) was made of the surface roughness. The roughness profile (two dimensions) was determined from the largest valley depth deviation from the mean line within a given length of 2.6 mm. Five measurements were made for each specimen, and the mean of these measurements was considered the roughness profile of that specimen. The surface roughness data were expressed in micrometers and were analyzed by two-way repeated-measures ANOVA followed by Tukey post hoc multiple-comparison tests (α=0.05). The roughness

Table 2: *Mean Gloss (±SD) of the RBCs Before and After Brushing*[a]

RBC	Unbrushed, GU	Brushed, GU	Mean Decrease of Gloss
Admira Fusion X-tra	81.6 (2.2) A d	2.9 (0.7) B f	96.4%
Aura	82.5 (3.3) A d	14.5 (1.4) B d	82.4%
Filtek Bulk Fill Flowable	94.5 (0.9) A a	28.7 (6.4) B c	69.7%
Filtek Bulk Fill Posterior	87.4 (3.3) A bcd	52.7 (5.3) B b	39.7%
Filtek Supreme Ultra	90.2 (2.2) A ab	69.8 (4.2) B a	22.6%
Herculite	89.3 (2.1) A abc	29.9 (0.9) B c	66.5%
Mosaic	90.6 (1.7) A ab	75.7 (2.5) B a	16.4%
SDR flow+	89.5 (2.1) A ab	11.8 (2.4) B de	86.8%
Sonic Fill (set:1)	90.8 (2.6) A ab	23.1 (2.6) B c	74.6%
Sonic Fill (set:5)	89.6 (3.1) A ab	26.5 (1.0) B c	70.4%
Tetric EvoCeram Bulk Fill	83.3 (3.0) A cd	13.3 (1.8) B d	84.0%
Tetric EvoFlow Bulk Fill	93.1 (1.9) A ab	7.1 (2.1) B ef	92.4%

[a] *Uppercase letters compare roughness between unbrushed and brushed surfaces within the same RBCs ($p<0.05$). Lowercase letters compare RBCs within the same composite surface (unbrushed or brushed; $p<0.05$).*

profile (in micrometers) was analyzed by one-way ANOVA and Tukey post hoc multiple-comparison tests ($\alpha=0.05$).

Surface Loss (Wear) and Topographical Analysis

A noncontact optical profilometer (Proscan 2100, Scantron, Venture Way, Taunton, UK) with an S11/03 sensor that has a resolution of 0.012 μm was used to determine the surface wear caused by brushing. A 1-mm × 0.5-mm central area of the specimen that included both brushed and unbrushed surfaces was scanned for this analysis. A step size of 0.01 mm (number of steps: 100) was set for the x-axis, and a step size of 0.05 mm (number of steps: 10) was set for the y-axis. The depth of the brushed surface was assessed using the two-point height tool of the Proscan Application Software v.2.0.17, using the unbrushed surface as a reference. The mean height difference between the unbrushed and brushed areas of the specimen was calculated to obtain the surface loss. Data were exported to software (OriginPro 2017, OriginLab, Northampton, MA, USA), and representative images of the scanned surfaces were produced. The surface loss was expressed in micrometers and was analyzed using one-way ANOVA followed by Tukey post hoc multiple-comparison tests ($\alpha=0.05$). Images of the specimen surfaces comparing the unbrushed and the brushed sides (1500×) were also obtained using a digital microscope (KH-1300, Hirox Co Ltd, Tokyo, Japan).

Correlation Coefficient

Pearson correlation tests ($\alpha=0.05$) were used to identify if there was any correlation between gloss and surface roughness, surface loss and percentage of gloss decrease after brushing, and surface loss and surface roughness after brushing.

RESULTS

Gloss Retention

The mean gloss data before and after brushing and the mean percentage of gloss decrease are reported in Table 2. Before brushing, the initial gloss of the materials that had been light cured against the Mylar ranged from 81.6 GU (Admira Fusion X-tra) to 90.6 (Mosaic Enamel). After brushing, the gloss decreased for all the RBCs tested ($p<0.05$). The mean percentage of gloss decrease ranged from 96.4% (Admira Fusion X-tra) to 16.4% (Mosaic Enamel). Mosaic Enamel and Filtek Supreme Ultra showed the greatest gloss retention after brushing, with only a 16.4% and 22.6% decrease, respectively ($p<0.05$). There was no statistical difference between the specimens made on the two sonication settings (1 and 5) for Sonic Fill 2 ($p\geq0.05$).

Surface Roughness and Roughness Profile

Table 3 shows the mean surface roughness before and after brushing and the magnitude of the increase in surface roughness after brushing compared with the unbrushed side. Before brushing, the initial surface roughness ranged from 0.08 μm for Filtek Bulk Fill Flowable to 1.14 μm for Tetric EvoCeram Bulk Fill. After brushing, the surface roughness showed an increase for all RBCs ($p<0.05$), with values ranging from a low of 0.99 μm (Filtek Bulk Fill Flowable) to 2.67 μm (Filtek Bulk Fill Posterior). Filtek Bulk Fill Posterior showed the

Table 3: *Mean (±SD) Surface Roughness (Sa) of the Unbrushed and Brushed Surfaces of the RBCs*[a]

RBC	Unbrushed, μm	Brushed, μm	Times Increase of Sa
Admira Fusion X-tra	0.77 (0.32) A abc	2.05 (0.15) B bc	2.7
Aura	0.98 (0.55) A ab	1.67 (0.21) B c	1.7
Filtek Bulk Fill Flowable	0.08 (0.01) A e	0.99 (0.09) B e	12.4
Filtek Bulk Fill Posterior	0.56 (0.17) A bcde	2.67 (0.45) B a	4.8
Filtek Supreme Ultra	0.38 (0.09) A cde	2.36 (0.36) B ab	6.2
Herculite	0.59 (0.19) A bcd	1.68 (0.18) B c	2.8
Mosaic	0.15 (0.02) A de	1.14 (0.25) B de	7.6
SDR flow+	0.19 (0.04) A de	1.59 (0.07) B cd	8.4
Sonic Fill (set: 1)	0.42 (0.05) A cde	1.72 (0.10) B c	4.1
Sonic Fill (set: 5)	0.46 (0.15) A cde	1.67 (0.12) B c	3.6
Tetric EvoCeram Bulk Fill	1.14 (0.29) A a	2.20 (0.11) B ab	1.9
Tetric EvoFlow Bulk Fill	0.16 (0.02) A de	1.99 (0.15) B bc	12.4

[a] *Upper case letters compare roughness between unbrushed and brushed surfaces within the same RBCs ($p<0.05$). Lower case letters compare RBCs within the same composite surface (unbrushed or brushed) ($p<0.05$).*

greatest surface roughness after brushing, although it was not statistically different from the results for Filtek Supreme Ultra and Tetric EvoCeram Bulk Fill. On the other hand, Filtek Bulk Fill Flowable had the least surface roughness after brushing (although not statistically different from Mosaic Enamel). This was despite the fact that the surface roughness of Filtek Bulk Fill Flowable increased 12.4× after brushing. No statistical differences were observed in the roughness between the specimens made on the two different sonication settings (1 and 5) of Sonic Fill 2 either before or after brushing ($p\geq0.05$).

The mean roughness profile data are reported in Table 4. Admira Fusion X-tra had the greatest roughness profile (3.38 μm), although this was not statistically different ($p\geq0.05$) from Tetric EvoCeram Bulk Fill (2.84 μm), Filtek Bulk Fill Flowable (2.44 μm), and Aura (2.38 μm). Mosaic Enamel had the lowest roughness profile (0.92 μm), which was not statistically different from Filtek Bulk Fill Posterior (1.24 μm). There were no statistical differences observed between the specimens made using the two sonication settings (1 and 5) of Sonic Fill 2 ($p\geq0.05$).

Table 4: *Mean (±SD) Roughness Profile (Rv) of the RBCs After Brushing*[a]

RBC	Rv, μm
Admira Fusion X-tra	3.38 (1.23) a
Aura	2.38 (0.33) ab
Filtek Bulk Fill Flowable	2.44 (0.45) ab
Filtek Bulk Fill Posterior	1.24 (0.12) cd
Filtek Supreme Ultra	1.99 (0.47) bc
Herculite	2.02 (0.27) bc
Mosaic	0.92 (0.14) d
SDR flow+	2.04 (0.19) bc
Sonic Fill (set: 1)	2.21 (0.31) bc
Sonic Fill (set: 5)	2.08 (0.23) bc
Tetric EvoCeram Bulk Fill	2.84 (0.48) ab
Tetric EvoFlow Bulk Fill	2.28 (0.31) b

[a] *Different letters indicate significant differences among RBCs ($p<0.05$).*

Surface Loss (Wear) and Topographical Analysis

The mean surface height loss values are reported in Table 5. Admira Fusion X-tra had the greatest surface loss (3.51 μm) compared with the other RBCs ($p<0.05$). The surface loss ranged from a low of 0.75 μm (Filtek Supreme Ultra) to 3.51 μm (Admira Fusion X-tra). No statistical differences were observed between specimens made using the

Table 5: *Mean (±SD) Loss in Height of the RBCs*[a]

RBC	Height Loss, μm
Admira Fusion X-tra	3.51 (0.55) a
Aura	2.33 (0.63) b
Filtek Bulk Fill Flowable	1.19 (0.18) ef
Filtek Bulk Fill Posterior	0.82 (0.18) f
Filtek Supreme Ultra	0.75 (0.14) f
Herculite	1.39 (0.19) def
Mosaic	0.88 (0.13) f
SDR flow+	1.97 (0.21) bcd
Sonic Fill (set: 1)	1.15 (0.13) ef
Sonic Fill (set: 5)	1.23 (0.09) ef
Tetric EvoCeram Bulk Fill	2.23 (0.36) bc
Tetric EvoFlow Bulk Fill	1.56 (0.31) cde

[a] *Different letters indicate significant differences among the RBCs ($p<0.05$).*

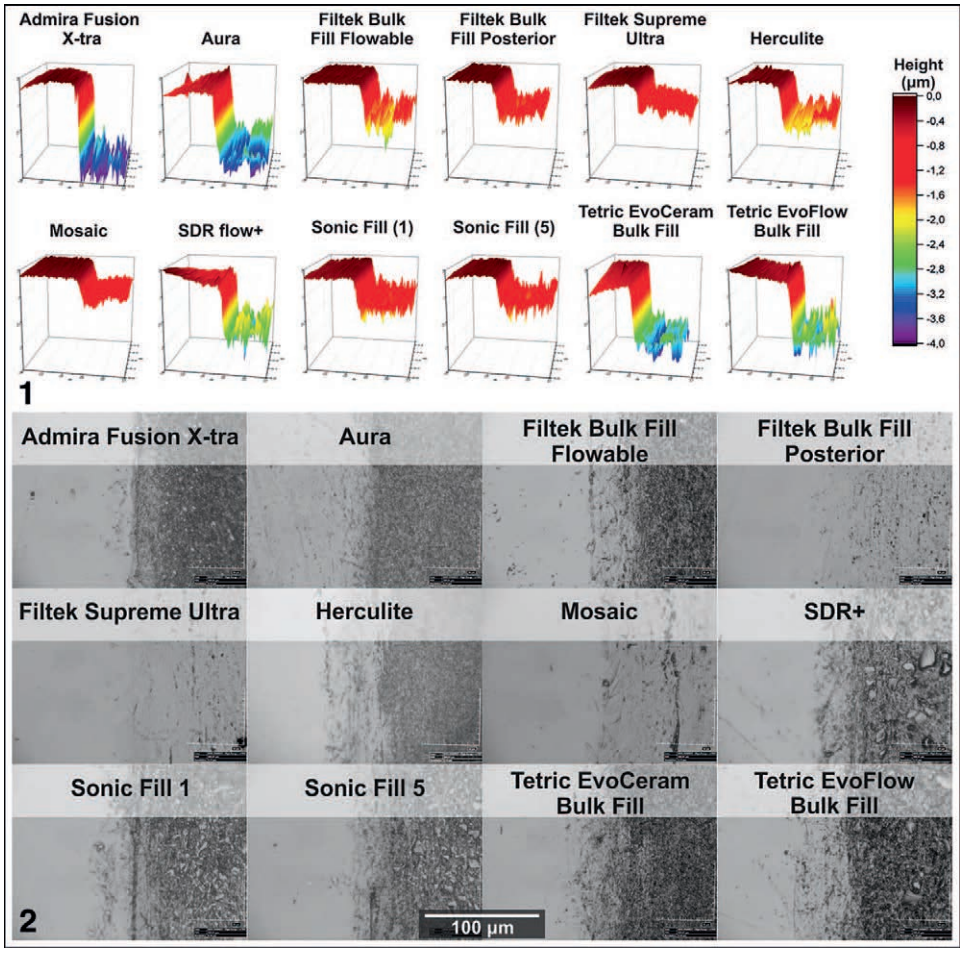

Figure 1. *Loss in surface height (µm) of the RBCs, comparing the nonbrushed (reference surfaces) and the surfaces after brushing.*

Figure 2. *Surfaces of the RBCs before (left side) and after (right side) brushing.*

two sonication settings (1 and 5) of Sonic Fill 2 ($p \geq 0.05$). Figures 1 and 2 are representative images of the values described in Table 5 and Table 3, respectively. In Figure 1, the deepest areas, represented by the dark blue and purple colors, were observed in Admira Fusion X-tra, Aura, Tetric EvoCeram Bulk Fill, and Tetric EvoFlow Bulk Fill (Figure 1). Figure 2 shows representative microscopy images of all the RBCs on the unbrushed (left) and the brushed sides (right). After brushing, the filler particles of some RBCs were exposed, which can be seen in the images of Admira Fusion X-tra, SDR+, Sonic Fill 2, and Tetric EvoFlow Bulk Fill. The image of SDR flow+ after brushing shows filler particles that were greater than 10 µm in size.

Correlation Coefficient

Figure 3 shows that the decrease in gloss after the toothbrushing for most of the samples tested was correlated with the increase in surface roughness (inverse linear correlation, $p<0.05$; $R^2=0.5504$). Figure 4 shows the positive correlation ($p<0.05$; $R^2 = 0.4617$) between the percentage of gloss decrease and the surface loss (wear of the material). Figure 5 shows that there was a weak interaction between surface roughness and surface loss ($p<0.05$; $R^2=0.0802$).

DISCUSSION

RBCs have become the material of choice for direct restorations and are widely used in dental practice.[5] Clinically, maintaining a smooth surface on the RBC is important because it may reduce plaque retention, surface discoloration, tissue inflammation, and secondary caries; improve the esthetics; and potentially add to patient comfort.[22,23]

In this study, toothbrushing with a dentifrice decreased the surface gloss and increased the surface roughness for all tested RBCs, thus rejecting both the first and the second null hypotheses. The differences in roughness profile and wear after brushing among the RBCs meant that the third and fourth null hypotheses were also rejected.

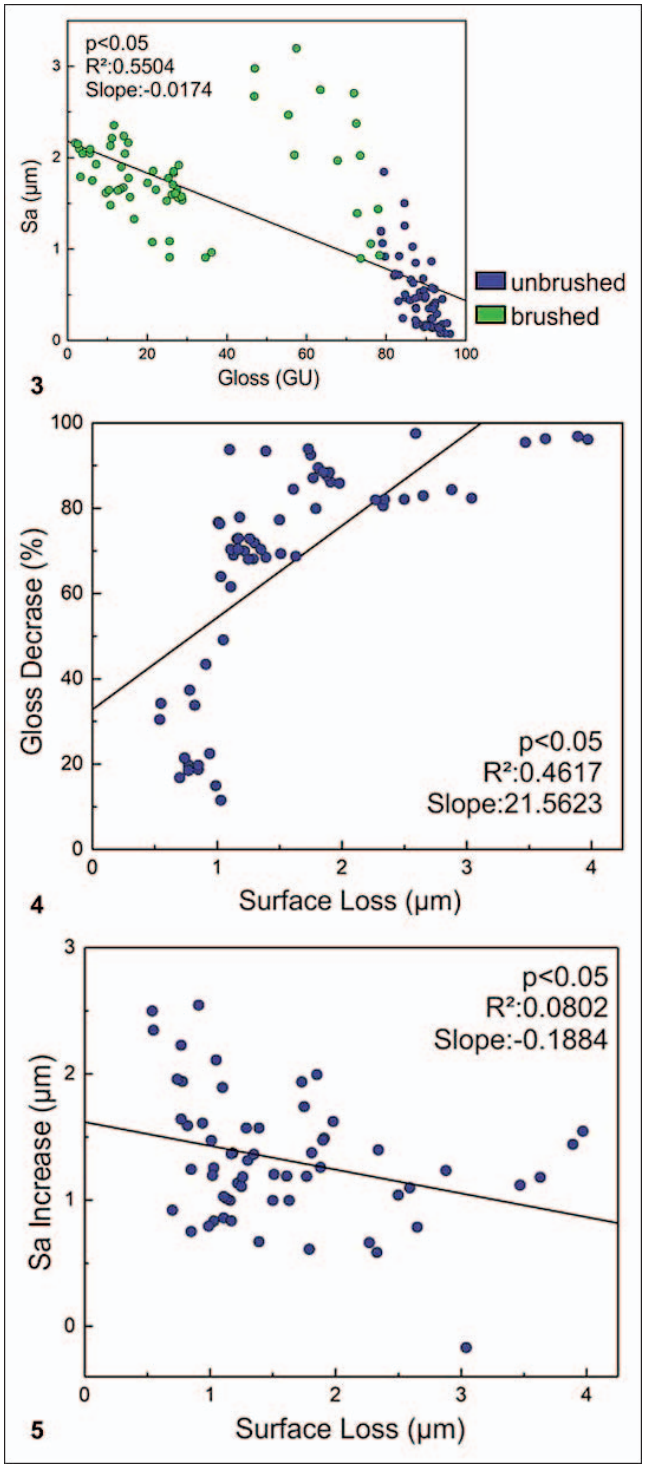

Figure 3. *Scatter graph showing a moderate negative correlation between RBC surface roughness (Sa) and gloss (GU) for unbrushed and brushed side (*p *and* R^2 *values, Pearson correlation test).*

Figure 4. *Scatter graph showing a positive correlation between RBC surface gloss decrease and surface loss after brushing (*p *and* R^2 *values, the Pearson correlation test).*

Figure 5. *Scatter graph showing a weak negative correlation between RBCs surface roughness (Sa) and surface loss (µm).*

Among many factors, the gloss can be affected by the particle size, chemical heterogeneity, and surface defects in the RBC.[22,24] Two of the RBCs tested, Admira Fusion X-tra (a nanohybrid Ormocer, organically modified ceramics) and Tetric EvoFlow Bulk Fill (a flowable bulk-fill composite), had more than a 90% decrease in gloss. The manufacturer recommends that Admira Fusion X-tra be used for class I, II, and V restorations and not in esthetic areas such as class III and IV, where the loss in gloss would be more important to the patient. Mosaic Enamel exhibited the greatest gloss retention (16.4% gloss decrease) followed by Filtek Supreme Ultra (22.6% gloss decrease); these RBCs are conventional nanohybrid and nanofilled composites, respectively. Herculite Ultra, also a conventional composite, had low gloss retention (66.5% gloss decrease). This RBC includes nanohybrid filler particles and was expected to have better gloss retention due to the spherical-shaped filler particles.[23] However, since the gloss decreased after 2 years of simulated toothbrushing, restorations may require repolishing or even replacement, to meet the esthetic requirements of the patient.[25]

It is claimed that the larger size of the monomer molecules used in the Ormocer would reduce polymerization shrinkage and wear and leach fewer monomers.[26] However, this RBC exhibited the greatest surface loss, a large increase in surface roughness, a rough surface profile after brushing, as well as a large decrease in surface gloss. Another study has also reported low gloss retention and an increase in surface roughness for Admira Fusion X-tra, even after only 5000 reciprocal strokes.[19]

Some studies report that the surface roughness of composites is a key factor in biofilm formation,[17,27] and this increases the risk of caries around dental restorations.[28] Conversely, other studies have failed to report any significant relationship between the surface roughness and an increased biofilm formation.[16] However, this result may be due to the difficulty in standardizing the polishing procedure[28] or by the prolonged bacterial incubation that was used in the study, in which new bacteria adhered to the biofilm and not to the surface of the RBC that was being tested.[17] Another study showed that the surface topography might be considered more important for *Streptococcus mutans* biofilm formation than surface roughness, and according to the same authors, deeper and larger depressions may provide a more favorable region for bacterial colonization and biofilm formation because bacterial colonies are more difficult to remove from a rough surface.[28] In

the present study, the topography was analyzed both by the surface roughness and by the roughness profile. Considering the surface topography of the different specimens, it is suggested that there will be increased and more mature biofilm formation around brushed Admira Fusion X-tra, Aura, Tetric EvoCeram Bulk Fill, and Tetric EvoFlow Bulk Fill RBCs. However, further investigations regarding the formation of biofilm after brushing are required as many other factors such as the chemical composition and the surface free energy of the RBC may affect biofilm formation.[17]

Previous studies have considered that bacterial adhesion should not occur below a profile roughness threshold of 0.2 μm.[29] According to this threshold, bacterial adhesion would be increased for all tested RBCs after toothbrushing (Table 3). However, this threshold was based on a profile roughness instead of the surface roughness measurement that was used in the present study. Also, a recent systematic review identified that a threshold could not reliably predict the bacterial adhesion.[30] This may make the comparison of the results of the present studies and this threshold unfeasible.

The roughness that patients could theoretically detect was also studied using a previously established profile roughness threshold of 0.5 μm.[31] All RBCs tested would present a roughness increase of more than this value after the simulated 2-year toothbrushing (Table 3), which may lead to the necessity of repolishing restorations, but again, this threshold was also reported in profile roughness and not surface roughness.

Although some flowable bulk-fill composites such as Filtek Bulk Fill Flowable can be placed in small posterior occlusal restorations, other flowable bulk-fill composites, such as SDR or Tetric EvoFlow Bulk Fill, are not recommended by the manufacturers to be placed in areas of occlusal loading, where a covering layer of a more wear-resistant RBCS is required.[4] More wear was expected for all the flowable bulk-fill composites, but this was not always the case. Instead, the flowable RBCs displayed intermediate results or even low results, such as those seen in Filtek Bulk Fill Flowable. However, this study evaluated only the wear of RBCs after toothbrushing, and the effect of the occlusal loading on these materials was not tested. Of the high-viscosity bulk-fill composites, Filtek Bulk-Fill posterior maintained the greatest gloss retention (39.7% of gloss decrease) and the least wear after toothbrushing.

Although the wear resistance may depend on the degree of conversion of the monomers,[33] these RBCs were well cured and stored for 24 hours before testing. It is expected that RBCs that have a lower filler loading may have greater wear because the resin matrix is less protected by the fillers and will be more readily removed.[32,33] However, Admira Fusion X-tra and Aura had greater wear, even though both were nanohybrid composites with 84% and 81% of filler loading, respectively. There was less wear on Filtek Bulk Fill Posterior, Filtek Supreme Ultra (nanofilled), and Mosaic Enamel (nanohybrid). Figures 1 and 2 also show a flatter surface for these RBCs compared with the other RBCs that were tested. Thus, the gloss retention and wear results reported in this study corroborate the theory that RBCs may differ after brushing due to the quality of silanization of the organic matrix,[13] as the gloss decrease or the amount of surface loss could not be predicted from the percentage filler loading.

The fifth null hypothesis was also rejected because a significant inverse correlation between gloss and surface roughness was observed. Other authors have also observed a similar inverse linear correlation between gloss and surface roughness after toothbrushing.[19,34] This occurs because the rougher the material, the more light is scattered on its surface, leading to a decrease in the gloss.[14] Also, a positive correlation between gloss decrease and surface loss and a negative correlation between surface roughness and surface loss was observed. However, these correlations were weak (R^2=0.46 and 0.08). This observation may be explained by the difference in the reflective index of the exposed filler particles, reducing the gloss retention relative to the wear of the resin composites. The whitening toothpaste used in the study had an RDA of 101 and can be considered to have a medium abrasiveness. This RDA can be compared with the abrasiveness of some prophylactic polishing pastes.[35] Thus, after an initial surface roughness increase, the toothbrushing may have caused some polishing of the surface of the RBCs, leading to a weak positive correlation between gloss decrease and surface loss and a weak negative correlation between surface roughness and surface loss.

The excellent results for Mosaic Enamel suggest this material can be successfully used for RBC restorations, with regard to the wear expected from toothbrushing. Also, regarding anterior esthetic restorations requirements, this material retained a high gloss value. Most of the high-viscosity bulk-fill RBCs achieved properties comparable with those of

conventional RBCs, except for Admira fusion X-tra, which was the most affected by toothbrushing. No difference between the sonication settings were observed for Sonic Fill 2 regarding the properties tested; however, further physical and mechanical properties should be studied to determine the best sonication setting.

CONCLUSIONS

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

1. Toothbrushing with a dentifrice reduced the gloss, increased the surface roughness, and caused surface loss for all RBCs tested.
2. There was a negative correlation between gloss and surface roughness.
3. Considering all the tested properties, Mosaic Enamel displayed excellent gloss retention, low surface profile roughness, and low wear; in contrast, Admira Fusion X-tra was the most affected by toothbrushing, exhibiting the greatest decrease in gloss, the greatest roughness, and the most wear.
4. The properties tested were product and not type dependent.

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Conflict of Interest

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

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Influence of Bleaching and Aging Procedures on Color and Whiteness of Dental Composites

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Clinical Relevance

Color changes after bleaching resin-based composites were clinically acceptable, while aging caused clinically perceptible color changes.

SUMMARY

Bleaching can cause perceptible color changes on resin-based composite (RBC) restorations that may not be stable with aging. The objective of this study was to evaluate color stability and whiteness variations of RBCs after bleaching and aging procedures. Discs (10 mm in diameter and 1 mm thick) of shades A2 and A3 were fabricated from two RBCs (Filtek Z250 and Filtek Z350 XT) and divided into three subgroups (for each composite and shade) (n=5) as follows: control (no bleaching), at-home bleaching, and in-office bleaching. All specimens underwent an accelerated artificial aging up to 450 KJ/m^2 and 900 KJ/m^2 in an aging chamber (Suntest XXL+). A spectroradiometer (SpectraScan PR-670) was used to obtain CIE $L^*a^*b^*$ coordinates. CIEDE2000 color difference (ΔE_{00}) and whiteness index for dentistry (WI_D) were used to evaluate color stability. Color and whiteness differences data were analyzed considering the 50:50% visual color difference thresholds (perceptibility [PT] and acceptability [AT]) and 50:50% whiteness thresholds (whiteness perceptibility [WPT] and whiteness acceptability [WAT]). Analysis of variance and Tukey tests (α=0.05) were used to statistically analyze the data. After bleaching, all specimens showed ΔE_{00} and ΔWI_D values below their corresponding acceptability thresholds (AT and WAT, respectively). After aging, L^* and WI_D values decreased while b^* values increased ($p \leq 0.05$), resulting in ΔE_{00} and ΔWI_D values above AT and WAT, respectively. Color changes after bleaching RBCs were clinically acceptable, while aging provoked clinically perceptible color changes.

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INTRODUCTION

Tooth bleaching, which is a noninvasive procedure, is among the most popular treatments to improve tooth color and appearance.[1] Numerous products and methods for tooth bleaching have been described in the literature.[2,3] The original concept of at-home bleaching (HB) used 10% carbamide peroxide gel in a customized tray with supervision and guidance of a dental clinician.[1] Nowadays, there are many vital tooth bleaching procedures, including HB, in-office bleaching (OB) and over-the-counter bleaching systems.[2-4] Different bleaching agents, concentrations, times of application, product format, and application have been reported.[2]

Bleaching agents (carbamide peroxide and hydrogen peroxide) used in tooth bleaching are able to provide perceptible color changes. At the same time, these products may produce color and surface alterations of resin-based composite (RBC) restorations.[5] Such changes are influenced by the type and concentration of bleaching agents and bleaching time,[5-7] the type of the RBC (composition and percentage of organic and inorganic phases),[5,6] and various abrasive/erosive procedures related to diet and oral hygiene.[8]

CIELAB color space and its associated CIELAB (ΔE^*_{ab}) and CIEDE2000 (ΔE_{00}) total color difference formulas are extensively used for color research in dentistry. In this sense, whiteness variations are commonly evaluated by means of total color differences or differences among one of the three axes that describe the CIELAB color space (ΔL^*: differences in lightness; Δa^*: differences in red-green axis; and Δb^*: differences in yellow-blue axis).[9,10]

However, whiteness of a material is adequately portrayed with a whiteness index. Some whiteness indexes based on the CIE 1931 XYZ color notation system have been used in dental research: the CIE whiteness index (WIC),[11] the whiteness index (WI),[12] and the optimized whiteness index (WIO).[13] The most recent published whiteness index for dentistry (WI_D) is based on CIELAB space and correlates to perception of tooth whiteness.[14]

According to the latest guidance on color measurements published by the International Organization for Standardization,[15] color stability after aging and staining (or after bleaching procedures) should be assessed on the basis of 50:50% acceptability (AT: ΔE^*_{ab}=2.7 and ΔE_{00}=1.8) and 50:50% perceptibility (PT: ΔE^*_{ab}=1.2 and ΔE_{00}=0.8) thresholds.[16] In this sense, if the total color difference measured before and after aging or staining is at or below PT, it represents an excellent match; if the difference is between PT and AT, it represents an acceptable match; and if the difference is above AT, it represents an unacceptable match. In addition, WI_D variations (ΔWI_D) can be assessed through comparison with 50:50% acceptability and 50:50% perceptibility thresholds for whiteness (WPT=0.61; and WAT=2.9, respectively) obtained in a cohort study with laypersons.[17]

Therefore, the purpose of this study was to evaluate the color stability and whiteness of two RBCs subjected to dental bleaching procedures (at-home bleaching and in-office bleaching) and artificial accelerated aging (450 KJ/m^2 and 900 KJ/m^2), testing the hypothesis that both bleaching and aging procedures produce color changes and whiteness variations of RBCs greater than the 50:50% acceptability thresholds (AT and WAT).

METHODS AND MATERIALS

Specimen Preparation

A total of 180 disc-shaped specimens (10 mm in diameter and 1 mm thick) of shades A2 and A3 from two dental RBCs (Filtek Z250 [Z2] and Filtek Z350 XT [Z3]; Table 1) were fabricated using polytetrafluoroethylene molds. Thus, 45 specimens (n=5) were fabricated from each RBC-shade combination and divided into nine experimental treatments (Table 2).

RBCs were packed into the mold and pressed between two glass slides lined with polyester film (Mylar, DuPont, Wilmington, DE, USA). The specimens were light activated (40 seconds; 20 s/side) using a light curing unit (Radii; SDI, Bayswater, Victoria, Australia) at 1200 mW/cm^2. Specimens were kept in a dark environment at 37°C before testing. Combinations of experimental treatments, according to different bleaching and accelerated aging procedures, are described in Table 2.

Bleaching Procedures

Bleaching products used in the present study are described in Table 1. Two bleaching procedures (OB and HB) were performed according to manufacturer's instructions. As OB treatments usually need three applications of the product, the bleaching gel was applied on the specimens for 15 min/d for three consecutive days. HB treatments usually last for 3 weeks; therefore, the bleaching gel was applied on the specimens for 2 h/d for 21 consecutive days. After each application, bleaching products were washed out and specimens were stored in 37°C distilled water. All bleaching procedures were performed by

Table 1: *Description of the Materials used in this Study*

Material	Composition[a]	Manufacturer
Z2-Filtek Z250 (Z2.2- shade A2; and Z2.3- shade A3)	Micro-hybrid dental restorative resin composite; *Organic matrix:* Bis-GMA, UDMA, Bis-EMA, and TEGDMA; *Filler particles:* SiO_2-ZrO_2 particles (0.01-3.5 μm), (60% by vol.)	3M ESPE (St Paul, MN, USA)
Z3-Filtek Z350 XT (Z3.2- shade A2; and Z3.3- shade A3)	Nanocomposite; *Organic matrix:* Bis-GMA, UDMA, Bis-EMA, TEGDMA. and PEGDMA; *Filler particles:* Non-agglomerated/ non-aggregated; nanosilica (5-20 nm) and zirconia (4-11 nm) fillers and aggregated SiO_2-ZrO_2 nanoclusters (0.6-10 μm), (63.3% by vol. and 78.5% by weight)	3M ESPE
Whiteness HP Blue 35%	In-office Bleaching product (35% hydrogen peroxide gel)	FGM Dental Products (Joinville, SC, Brazil)
White Class 6%	At-home Bleaching product (6% hydrogen peroxide gel)	FGM Dental Products

Abbreviations: Bis-GMA - bisphenol A-glycidyl methacrylate; UDMA - urethane dimethacrylate; Bis-EMA - bisphenol A diglycidyl methacrylate ethoxylated; TEGDMA - triethylene glycol dimethacrylate; PEGDMA - Poly(ethylene glycol) dimethacrylate
[a] *Data provided by manufacturers.*

one experienced dentist. Specimens not bleached serve as the control group.

Accelerated Aging Procedures

Specimens were subjected to artificial accelerated aging (Suntest XXL+, Ametek Atlas, Mount Prospect, IL, USA) according to International Organization for Standardization (ISO) 4892-2[18] and ISO 7491[19] standards. The artificial aging cycle consisted of 102 minutes of light exposure and 18 minutes of water spraying under artificial daylight (CIE D65 illuminant) at 38°C±3°C constant temperature and 50%±10% relative humidity, with a black panel temperature of 65°C and irradiance control in the 300 to 400 nm interval of 60 W/m^2.[20] The aging cycles were repeated in 150 KJ/m^2 increments until switch-off criteria of 450 KJ/m^2 (T1: estimating 1 year of aging) and 900 KJ/m^2 (T2: estimating 2 years of aging) total dosage levels were achieved.[21] For all OB and HB groups, accelerated aging was performed after bleaching procedures. Specimens that were not aged (T0) served as controls.

Color Measurements

A noncontact spectroradiometer ([SP], SpectraScan PR-670, Photo Research, Chatsworth, CA, USA) was used to obtain the spectral reflectance (380 to 780 nm; interval of 2 nm) from all RBC specimens. A white ceramic background (L^*=94.2, a^*=1.3, b^*=1.7) was used for color measurements, while a black ceramic background (L^*=3.1, a^*=0.7, b^*=2.4) was used for WI_D calculations. A coupling agent (refractive index n≈1.5) was used as contact media between specimen and background.[22-24]

As reported in previous studies,[25,26] since the SP field of measurement is 1°, the specimens were placed 35 cm away from SP, on a 45° tilted base and under constant illumination (CIE D65 standard illuminant). Illuminating/measuring configuration corresponds to CIE d/0°.[22,27,28] Three short-term measurements without replacement were performed for each specimen. The results for each specimen and background were averaged over the three measurements. CIELAB color coordinates for all specimens were calculated according to CIE D65 Standard Illuminant and CIE 2° Standard Colorimetric Observer.[29]

Color Differences

Computations for the CIEDE2000 color difference (ΔE_{00}) metric were done according to the following equation[29,30]:

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

where $\Delta L'$, $\Delta C'$, and $\Delta H'$ are the differences in each

Table 2: *Experimental Treatments Used in the Study*

Acronym	Description
CT0	Control group at baseline
CT1	Control group after chromatic aging (450 KJ/m^2)
CT2	Control group after chromatic aging (900 KJ/m^2)
OBT0	In-office bleaching at baseline
OBT1	In-office bleaching after chromatic aging (450 KJ/m^2)
OBT2	In-office bleaching after chromatic aging (900 KJ/m^2)
HBT0	At-home bleaching at baseline
HBT1	At-home bleaching after chromatic aging (450 KJ/m^2)
HBT2	At-home bleaching after chromatic aging (900 KJ/m^2)

Table 3: *Mean and Standard Deviation Values of CIELAB Color Coordinates and Whiteness Index (WI_D) Followed by the Statistical Groupings for Specimens of Filtek Z250 Shade A2 (Z2.2)* [a]

Groups	CIELAB Color Coordinates			WI_D
	L^*	a^*	b^*	
Z2.2-CT0	68.54 ± 0.59 aA	–1.10 ± 0.03 bB	9.97 ± 0.32 cB	26.62 ± 0.36 aA
Z2.2-CT1	66.02 ± 0.43 bA	–1.02 ± 0.14 bB	19.06 ± 0.73 bA	15.14 ± 1.12 bA
Z2.2-CT2	64.86 ± 0.30 cA	–0.33 ± 0.24 aA	22.12 ± 0.33 aB	9.58 ± 0.85 cA
Z2.2-OBT0	67.67 ± 0.29 aB	–0.73 ± 0.14 bA	11.02 ± 0.65 cA	24.15 ± 0.91 aB
Z2.2-OBT1	66.41 ± 0.33 bA	–0.83 ± 0.15 bA	20.43 ± 0.46 bA	13.38 ± 0.95 bA
Z2.2-OBT2	65.41 ± 0.82 cA	–0.31 ± 0.16 aA	23.06 ± 0.30 aA	8.78 ± 0.81 cA
Z2.2-HBT0	68.27 ± 0.28 aA	–0.62 ± 0.03 bA	11.23 ± 0.39 cA	23.99 ± 0.40 aB
Z2.2-HBT1	65.90 ± 1.12 bA	–0.97 ± 0.30 cA	19.97 ± 0.68 bA	13.97 ± 1.96 bA
Z2.2-HBT2	64.54 ± 0.39 cA	–0.26 ± 0.17 aA	22.99 ± 0.23 aA	8.30 ± 0.60 cA

Abbreviations: C, control; HB, at-home bleaching; OB, in-office bleaching; T0, no aging; T1, chromatic aging procedure (450 KJ/m²) equivalent to 1 year; T2, chromatic aging procedure (900 KJ/m²) equivalent to 2 years.
[a] *CIELAB color coordinates and WI_D values using a black background ($p \leq 0.05$; two-way analysis of variance). Different lowercase letters show statistical differences for mean values (color parameters) between different aging procedures (T0, T1, and T2) within the same bleaching procedure (C, OB, or HB) (column). Different capital letters show statistical differences for mean values (color parameters) between different bleaching procedures (C, OB, and HB) within the aging procedures (T0, T1, or T2) (column).*

parameter (lightness, chroma, and hue, respectively) for a pair of specimens using CIEDE2000. The weighting functions (S_L, S_C, and S_H) adjust the total color difference for variation in the location of the color difference pair in L^*, a^*, b^* coordinates. The parametric factors (K_L, K_C, and K_H) are correction terms for experimental conditions. Finally, a rotation function (R_T) accounts for the interaction between chroma and hue differences in the blue region.[29,30] To calculate the CIEDE2000 color difference formula, discontinuities due to mean hue computation and hue-difference computation were taken into account.[31]

Color differences were finally evaluated through comparisons with the 50:50% color difference thresholds (PT=0.81 ΔE_{00} units and AT=1.77 ΔE_{00} units) for tooth-colored restorative materials, established in a prospective multicenter research project.[16]

Whiteness Index for Dentistry

The Whiteness Index for Dentistry (WI_D) is a CIELAB-based index with a linear formulation. WI_D values were obtained according to the following equation:[14]

$$WI_D = 0.511L^* - 2.324a^* - 1.100b^* \qquad (2)$$

WI_D variations (ΔWI_D) can be assessed through comparison with recently published data on acceptability and perceptibility thresholds for whiteness obtained in a cohort study with laypersons.[17] The 50:50% perceptibility level was determined at 0.61 ΔWI units (WPT=0.61) while 50:50% acceptability level was determined at 2.90 ΔWI_D units (WAT=2.90).[17]

Statistical Analysis

The color parameters L^*, a^*, b^* and the WI_D values were statistically evaluated. Two-way analysis of variance (ANOVA) was used to evaluate significance for the factors: bleaching and accelerated aging. The Tukey post hoc test was used to identify differences between groups. A global significance level of 95% was used.

RESULTS

CIELAB color coordinates (L^*, a^* and b^*) and WI_D values for Z2.2, Z2.3, Z3.2, and Z3.3, before and after bleaching and with different accelerated aging procedures are shown in Tables 3 through 6. As the time of aging increased, the mean values of L* and WI_D decreased ($p \leq 0.05$) (Tables 3 through 6).

CIEDE2000 total color difference (ΔE_{00}) values between two different bleaching treatments on the same composite/shade group for each aging protocol are shown in Figure 1. Comparing different bleaching treatments at T0, T1, and T2, all values of color difference (ΔE_{00}) were below AT (acceptable match). Color differences below PT (excellent match) were shown by the following comparisons: Z2.2 (T2), Z2.3 (T0 and T2), Z3.2 (T0 and T2), and Z3.3 (T0 and T1) (Figure 1).

ΔE_{00} values between two different aging procedures on the same composite/shade group for each bleaching treatment are shown in Figure 2. Considering different aging procedures, the comparison

Table 4: *Mean and Standard Deviation Values of CIELAB Color Coordinates and Whiteness Index (WI_D) Followed by the Statistical Groupings for Specimens of Filtek Z250 Shade A3 (Z2.3)* [a]

Groups	CIELAB Color Coordinates			WI_D
	L^*	a^*	b^*	
Z2.3-CT0	66.38 ± 0.36 aA	–0.35 ± 0.04 cB	14.51 ± 0.22 cA	18.78 ± 0.39 aA
Z2.3-CT1	64.35 ± 0.79 bA	0.21 ± 0.26 bA	21.14 ± 2.81 bAB	9.13 ± 2.93 bA
Z2.3-CT2	62.40 ± 0.11 cC	0.43 ± 0.09 aA	23.74 ± 0.16 aA	4.78 ± 0.36 cB
Z2.3-OBT0	65.36 ± 0.19 aB	–0.23 ± 0.04 bA	14.27 ± 0.28 cA	18.23 ± 0.32 aA
Z2.3-OBT1	63.43 ± 0.25 bA	–0.52 ± 0.09 cB	21.50 ± 0.24 bA	9.96 ± 0.48 bA
Z2.3-OBT2	62.91 ± 0.33 cB	0.13 ± 0.11 aB	23.64 ± 0.31 aA	5.84 ± 0.52 cA
Z2.3-HBT0	65.45 ± 0.24 aB	–0.20 ± 0.05 bA	14.08 ± 0.15 cA	18.42 ± 0.34 aA
Z2.3-HBT1	64.34 ± 1.44 abA	–0.62 ± 0.26 cB	20.35 ± 0.89 bB	11.93 ± 2.05 bA
Z2.3-HBT2	63.39 ± 0.19 bA	0.14 ± 0.10 aB	23.66 ± 0.34 aA	6.04 ± 0.53 cA

Abbreviations: C, control; HB, at-home bleaching; OB, in-office bleaching; T0, no aging; T1, chromatic aging procedure (450 KJ/m^2) equivalent to 1 year; T2, chromatic aging procedure (900 KJ/m^2) equivalent to 2 years.
[a] CIELAB color coordinates and WI_D values using a black background ($p \leq 0.05$; two-way analysis of variance). Different lowercase letters show statistical differences for mean values (color parameters) between different aging procedures (T0, T1, and T2) within the same bleaching procedure (C, OB, or HB) (column). Different capital letters show statistical differences for mean values (color parameters) between different bleaching procedures (C, OB, and HB) within the aging procedures (T0, T1, or T2) (column).

between T1 and T2 showed CIEDE color differences below AT (acceptable match) for Z2.2, Z2.3, and Z3.3 within C and OB groups (Figure 2A,B) and for Z2.3 within HB group (Figure 2C). For all other groups, mean values of ΔE_{00} were above AT (unacceptable match) (Figure 2).

Figures 3 and 4 show mean and standard deviation values of WI_D after different bleaching treatments and accelerated aging procedures on Z2 and Z3, respectively. Comparing different aging procedures always showed significant differences ($p \leq 0.05$) (Tables 3 through 6). All values of ΔWI_D between bleaching treatments and control group for the same aging procedure were below WAT. Only some values of ΔWI_D were below WPT (Table 7).

DISCUSSION

The present study was designed to respond to relevant questions regarding color changes in RBC after bleaching and aging procedures. Although the CIELAB color difference metric is the most commonly used in dentistry, it has been demonstrated that the CIELAB color space assumes equal influence or weight for all color coordinates.[32] However, some studies[33,34] have suggested a discrepancy on sensitivity to the different color coordinates within the dental color space. In recent years, CIEDE2000[29]

Table 5: *Mean and Standard Deviation Values of CIELAB Color Coordinates and Whiteness Index (WI_D) Followed by the Statistical Groupings for Specimens of Filtek Z350 Shade A2 (Z3.2)* [a]

Groups	CIELAB Color Coordinates			WI_D
	L^*	a^*	b^*	
Z3.2-CT0	64.36 ± 0.68 aA	–1.31 ± 0.04 cC	7.67 ± 0.30 cA	27.49 ± 0.23 aA
Z3.2-CT1	63.13 ± 0.94 bA	–0.96 ± 0.50 bA	14.17 ± 3.92 bB	18.92 ± 3.62 bA
Z3.2-CT2	61.99 ± 0.51 cA	–0.67 ± 0.11 aA	20.57 ± 0.22 aB	10.60 ± 0.55 cA
Z3.2-OBT0	63.79 ± 0.43 aA	–1.15 ± 0.03 bB	7.74 ± 0.23 cA	26.77 ± 0.23 aB
Z3.2-OBT1	62.82 ± 0.61 bA	–1.33 ± 0.19 cB	16.85 ± 0.95 bA	16.65 ± 1.19 bB
Z3.2-OBT2	61.55 ± 0.34 cA	–0.79 ± 0.17 aA	20.60 ± 0.39 aB	10.63 ± 0.79 cA
Z3.2-HBT0	64.32 ± 0.18 aA	–1.07 ± 0.05 bA	7.13 ± 0.19 cB	27.51 ± 0.29 aA
Z3.2-HBT1	62.50 ± 0.21 bA	–1.49 ± 0.06 cC	16.63 ± 0.32 bA	17.11 ± 0.40 bB
Z3.2-HBT2	61.97 ± 0.34 cA	–0.53 ± 0.34 aA	21.28 ± 0.44 aA	9.48 ± 1.23 cA

Abbreviations: C, control; HB, at-home bleaching; OB, in-office bleaching; T0, no aging; T1, chromatic aging procedure (450 KJ/m^2) equivalent to 1 year; T2, chromatic aging procedure (900 KJ/m^2) equivalent to 2 years.
[a] CIELAB color coordinates and WI_D values using a black background ($p \leq 0.05$; two-way analysis of variance). Different lowercase letters show statistical differences for mean values (color parameters) between different aging procedures (T0, T1, and T2) within the same bleaching procedure (C, OB, or HB) (column). Different capital letters show statistical differences for mean values (color parameters) between different bleaching procedures (C, OB, and HB) within the aging procedures (T0, T1, or T2) (column).

Table 6: *Mean and Standard Deviation Values of CIELAB Color Coordinates and Whiteness Index (WI_D) Followed by the Statistical Groupings for Specimens of Filtek Z350 Shade A3 (Z3.3)* [a]

Groups	CIELAB Color Coordinates			WI_D
	L^*	a^*	b^*	
Z3.3-CT0	63.32 ± 0.44 aA	–1.07 ± 0.07 cB	10.61 ± 0.39 cA	23.16 ± 0.59 aA
Z3.3-CT1	62.05 ± 0.39 bA	–0.69 ± 0.07 bA	18.82 ± 0.35 bAB	12.61 ± 0.61 bA
Z3.3-CT2	61.43 ± 0.69 cAB	–0.47 ± 0.10 aB	21.20 ± 0.38 aB	9.16 ± 0.56 cA
Z3.3-OBT0	62.65 ± 0.23 aB	–0.92 ± 0.11 cA	10.50 ± 0.42 cA	22.60 ± 0.61 aA
Z3.3-OBT1	61.76 ± 0.52 bA	–0.87 ± 0.13 bA	19.45 ± 0.34 bA	12.19 ± 0.82 bA
Z3.3-OBT2	61.13 ± 0.48 cB	–0.41 ± 0.09 aB	21.65 ± 0.27 aB	8.38 ± 0.70 cA
Z3.3-HBT0	63.62 ± 0.49 aA	–0.77 ± 0.05 cA	10.62 ± 0.24 cA	22.63 ± 0.37 aA
Z3.3-HBT1	61.74 ± 0.31 cA	–0.81 ± 0.11 bA	19.13 ± 0.28 bB	12.40 ± 0.54 bA
Z3.3-HBT2	62.41 ± 0.54 bA	–0.17 ± 0.07 aA	22.70 ± 0.21 aA	7.32 ± 0.29 cB

Abbreviations: C, control; HB, at-home bleaching; OB, in-office bleaching; T0, no aging; T1, chromatic aging procedure (450 KJ/m^2) equivalent to 1 year; T2, chromatic aging procedure (900 KJ/m^2) equivalent to 2 years.
[a] CIELAB color coordinates and WI_D values using a black background ($p \leq 0.05$; two-way analysis of variance). Different lowercase letters show statistical differences for mean values (color parameters) between different aging procedures (T0, T1, and T2) within the same bleaching procedure (C, OB, or HB) (column). Different capital letters show statistical differences for mean values (color parameters) between different bleaching procedures (C, OB, and HB) within the aging procedures (T0, T1, or T2) (column).

color difference was increasingly implemented in color research. Studies suggested that ΔE_{00} shows a better correlation with visual perception than ΔE^*_{ab},[25,32,35,36] which is the reason for using the CIEDE2000 color difference metric in the present study. Nevertheless, there is only one study on color changes after dental bleaching using this metric.[8]

The use of total color differences, ΔE^*_{ab},[6,7,37-39] and ΔE_{00},[8] is very popular for evaluating color changes after dental bleaching. Yet, understanding and considering color differences within the visual perceptibility and acceptability thresholds is very relevant in clinical dentistry. Thus, studies on dental color should qualify their statistical analysis of the data associating them to PT and AT values. The most popular value for acceptable color difference used to be 3.3 ΔE^*_{ab} units.[6,7,37-39] Recently, an ISO standard[15] defined PT and AT values for tooth colored restorative materials. Such values were established in a prospective multicenter study[16] that used CIELAB (ΔE^*_{ab}) and CIEDE2000 (ΔE_{00}) total color difference metrics. The present study used PT and AT values reported in that work.[16]

Although it is common to use color difference formulas to evaluate color changes after bleaching procedures, a sole evaluation of color differences does not offer enough information on how color coordinates change. Thus, it is not adequate to compare whiteness values using only color difference metrics (ΔE^*_{ab} or ΔE_{00}). Although previous studies used other indexes for evaluating whiteness,[13,26,40,41] the present study used a recently published whiteness index (WI_D), which is based on CIELAB color space and was specifically designed for dentistry and dental applications.[14] WI_D was compared with other

Table 7: *Mean and Standard Deviation Values of Differences in Whiteness Index (ΔWI_D) for Specimens of Filtek Z250 (Z2.2 = Shade A2 and Z2.3 = Shade A3) and Filtek Z350 (Z3.2 = Shade A2 and Z3.3 = Shade A3) Between each Bleaching Procedure (OB or HB) and Control Group for the Same Aging Procedure (T0, T1, and T2)* [a]

Groups	ΔWI_D			
	Z2.2	Z2.3	Z3.2	Z3.3
OBT0-CT0	–2.47 ± 0.82	–0.55 ± 0.57[a]	–0.73 ± 0.44	–0.56 ± 0.69[a]
OBT1-CT1	–1.76 ± 0.63	0.83 ± 0.26	–2.26 ± 0.69	–0.42 ± 0.25[a]
OBT2-CT2	–0.80 ± 0.91	1.06 ± 0.72	0.02 ± 0.37[a]	–0.78 ± 0.64
HBT0-CT0	–2.63 ± 0.53	–0.36 ± 0.45[a]	0.02 ± 0.13[a]	–0.53 ± 0.61[a]
HBT1-CT1	–1.17 ± 0.48	2.80 ± 0.48	–1.81 ± 0.57	–0.21 ± 0.10[a]
HBT2-CT2	–1.28 ± 0.80	1.25 ± 0.52	–1.13 ± 0.46	–1.84 ± 0.41

Abbreviations: C, control; HB, at-home bleaching; OB, in-office bleaching; T0, no aging; T1, chromatic aging procedure (450 KJ/m^2) equivalent to 1 year; T2, chromatic aging procedure (900 KJ/m^2) equivalent to 2 years.
[a] Mean values below 50:50% perceptibility whiteness thresholds for laypersons (ΔWI_D=0.61).[17] Negative values indicate that bleaching procedure groups (OB or HB) showed lower values of WI_D compared with control group (C).

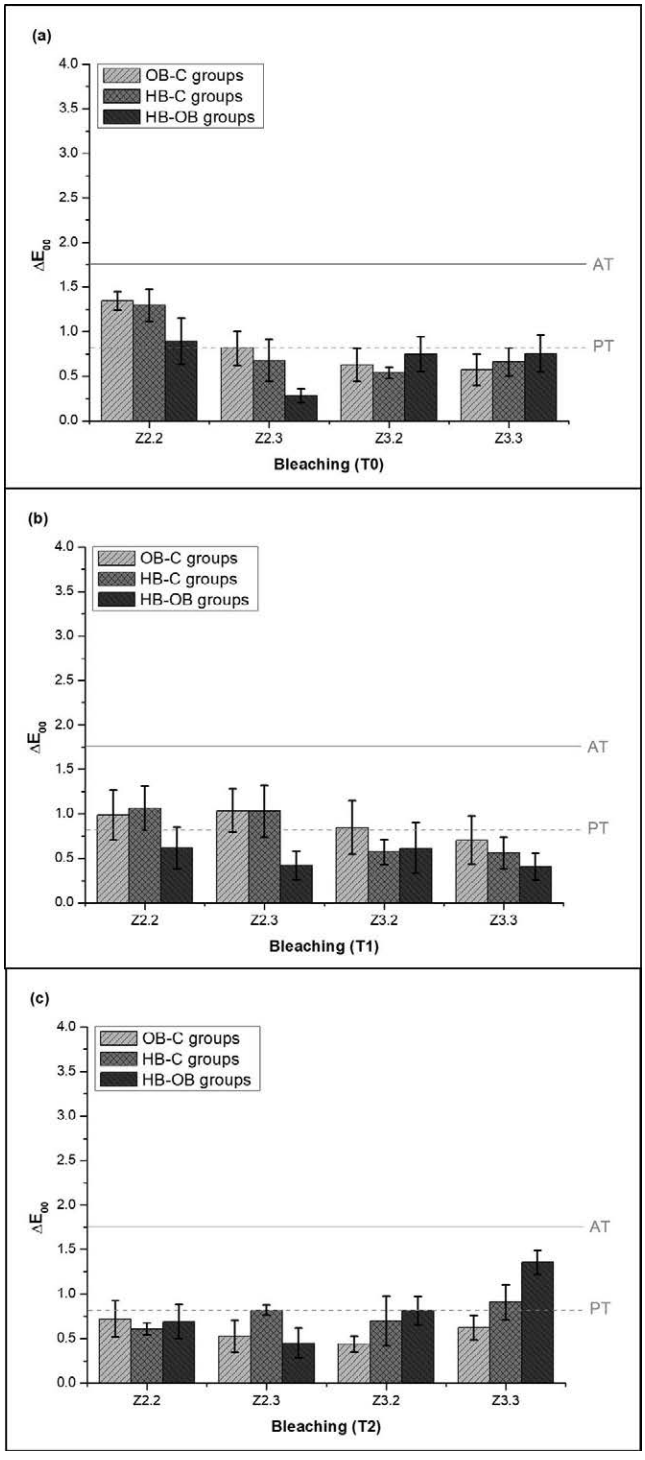

Figure 1. *Mean and standard deviation values of ΔE_{00} between two different bleaching treatments, in-office bleaching (OB) or at-home bleaching (HB) and control (C; no bleaching) on the same composite/shade group at (A): baseline (T0); (B): 450 KJ/m^2 estimating 1 year (T1); and (C): 900 KJ/m^2 estimating 2 years (T2). The horizontal lines at 1.77 and 0.81 (ΔE_{00} units) represent the 50:50% acceptability (AT) and the 50:50% perceptibility (PT) thresholds, respectively.*[16]

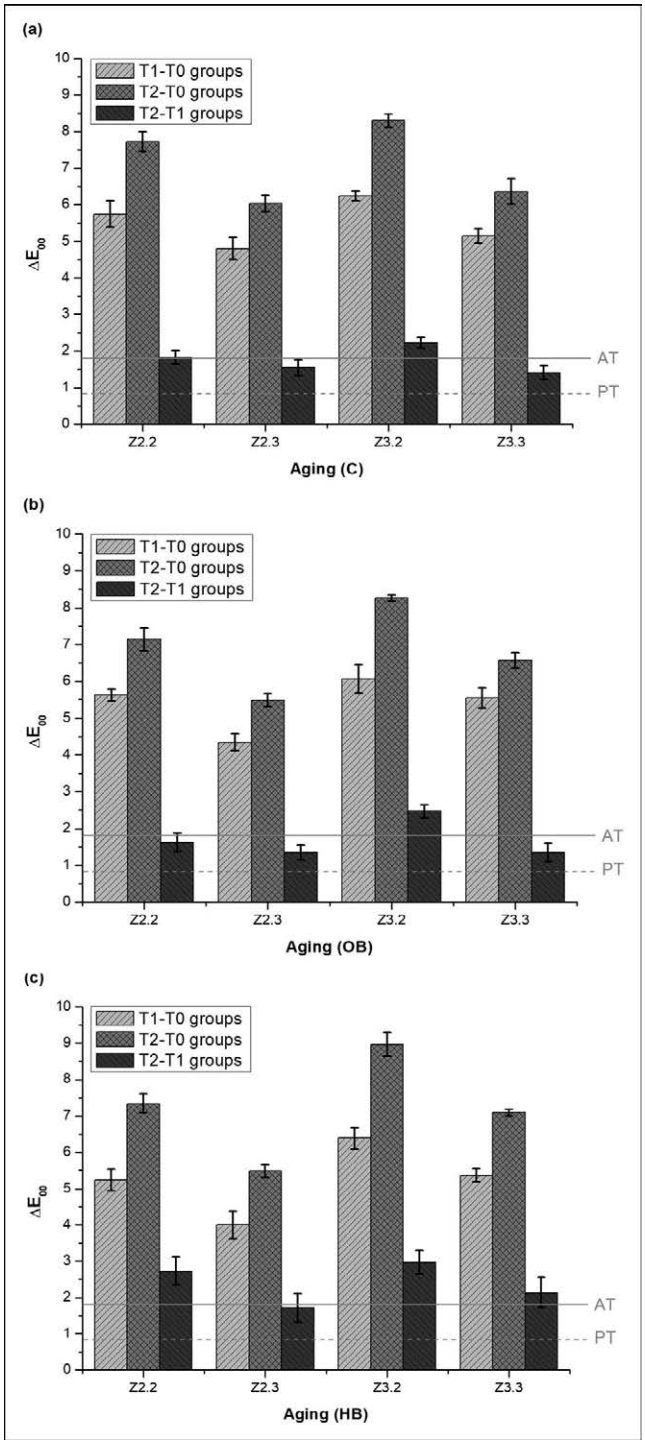

Figure 2. *Mean and standard deviation values of ΔE_{00} between two different chromatic aging procedures, 450 KJ/m^2 estimating 1 year (T1) or 900 KJ/m^2 estimating 2 years (T2) and the control (T0; no aging) on the same composite/shade for (A): control (C) groups; (B): in-office bleaching (OB) groups; and (**C**) at-home bleaching (HB) groups. The horizontal lines at 1.77 and 0.81 (ΔE_{00} units) represent 50:50% acceptability (AT) and 50:50% perceptibility (PT) thresholds, respectively.*[16]

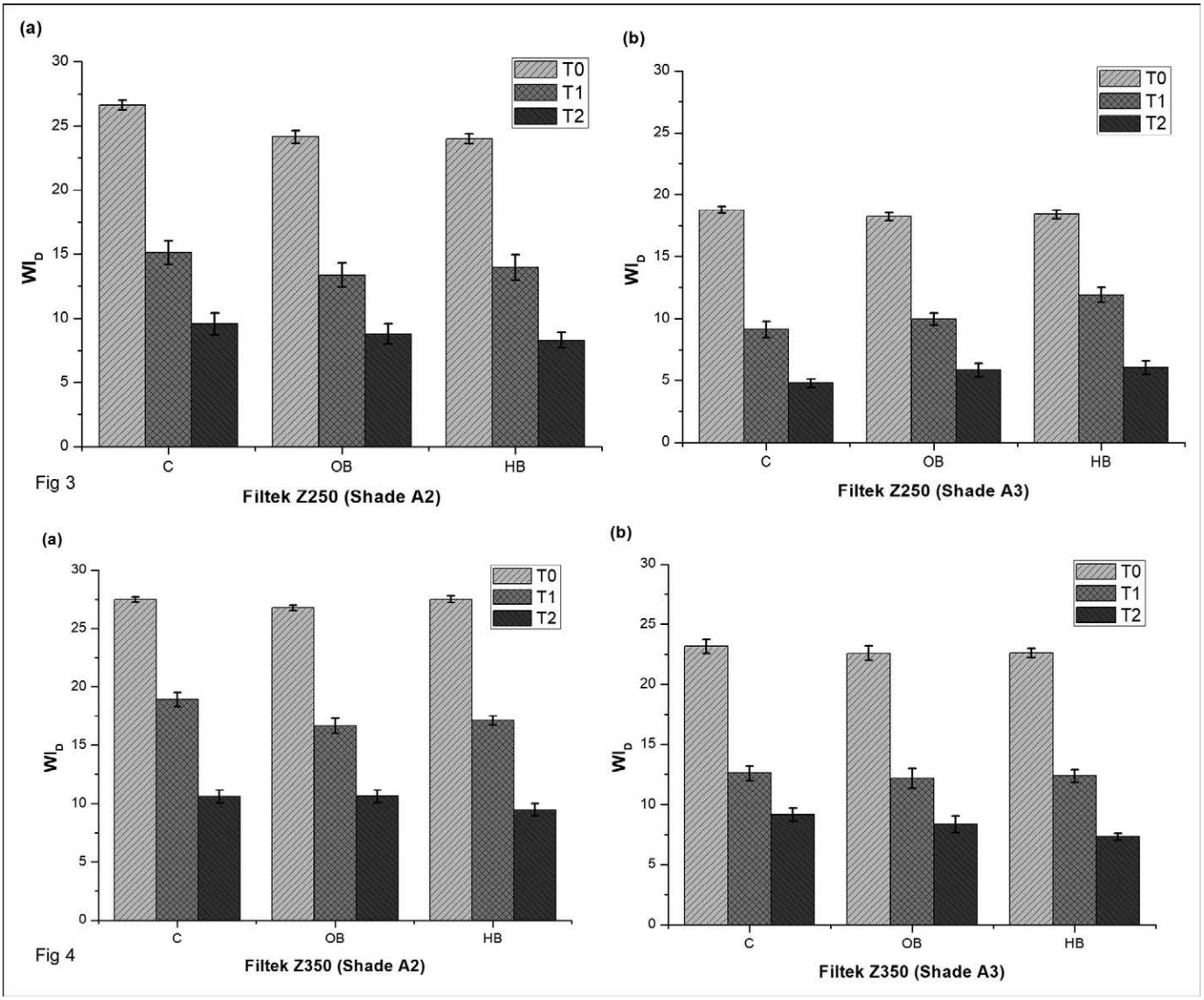

Figure 3. *Mean and standard deviation values of* WI_D *for Filtek Z250 shades (A): A2 and (B): A3 after different bleaching treatment (C, control; OB, in-office bleaching; and HB, at-home bleaching) and different chromatic aging procedures (T0, control; T1, after 450 KJ/m² estimating 1 year; and T2, after 900 KJ/m² estimating 2 years).*

Figure 4. *Mean and standard deviation values of* WI_D *for composite Filtek Z350 shades (A): A2 and (B): A3 after different bleaching treatment (C, control; OB, in-office bleaching]; and HB, at-home bleaching) and different chromatic aging procedures (T0, control; T1, after 450 KJ/m² estimating 1 year; and T2, after 900 KJ/m² estimating 2 years).*

whiteness indexes and showed a strong correlation to visual perception of tooth whiteness.[14]

The ΔWI_D values between different bleaching treatments (OB, HB, and control) for the same aging procedure (Table 7) were analyzed using values of whiteness thresholds (WPT and WAT) from a previous study[17] that found different WPT and WAT values for different observer groups (WPT = 0.61 and WAT = 2.90 for laypersons and WPT = 0.44 and WAT = 2.15 for dentists). Since patients are the evaluators (observers) of dental bleaching treatments, and the influence of observer population on color difference thresholds (PT and AT) has been previously reported,[16] the present study used WPT = 0.61 and WAT = 2.90.

Considering color differences and whiteness variations after bleaching of all experimental groups, the mean values of ΔE_{00} and ΔWI_D were below their corresponding acceptability value (AT=1.77 and WAT=2.90, respectively). Therefore, despite the

variations, the color changes and whiteness variations should be acceptable to observers (patients). Thus, the first part of the study hypothesis was not confirmed because bleaching procedures did not produce color changes and whiteness variations of RBCs greater than the 50:50% acceptability thresholds (AT and WAT).

The term "color stability" has been widely used in dental color research. A physical magnitude is stable when its variation along a process (in this case, bleaching or aging) is lower than the instrumental error. However, color is a psychophysical property; therefore, observer's interpretations (evaluations) should be considered. In this sense, color variations below the perceptibility threshold will not be detectable by a standard observer. Thus, color stability of a dental material, from an observer point of view, can be defined as a color variation (described by a color difference) lower than the PT. In most cases, the bleached nanocomposite showed color differences below PT values (Z3.3 after T0 and T1 and Z3.2 at time of aging) (Figure 1), suggesting this material presents appropriate color stability for the aging protocols evaluated in the present study.

Previous studies showed that bleaching treatments may affect the elution of monomers and other substances from RBCs.[42-44] The three-dimensional polymer network of RBCs, which consists of carbon-carbon (C-C)-single or (C-C)-double bonds, may react with oxidants like hydroxide peroxide, increasing unpolymerized monomers, additives and unspecific oxidative products release.[43,44] Complete polymerization of RBCs is still a challenge to overcome. Thus, the lower the conversion rate of an RBC, the more residual monomers can be eluted.[45] Both RBCs used in the present study were light activated under the same conditions (eg, activation time, light energy and distance), therefore they should not be considered as study variables. Yet, though the manufacturer did not provide the exact percentage of monomers, the three-dimensional polymer network is very similar for both RBCs. The main difference between the two RBCs is the amount of inorganic phase, which can influence the degree of conversion of an RBC. As is known, as the filler loading increases, the degree of conversion increases and the water sorption decreases. Thus, the greater color stability of the nanocomposite may be associated to its higher filler content (Table 1).

Previous studies showed that total bleaching time is more important than the concentration of the bleaching agent.[2,8] Although it was not an objective of the present study, the results did not show a significant difference between the effect of the different bleaching treatments on color of RBCs (Figure 1A).

Color stability of RBCs has been evaluated using an artificial aging chamber, exposing the specimens to ultraviolet light and elevated temperatures[20,21,46] or by immersing the RBC in various staining beverages.[47,48] The present study used an artificial aging procedure to simulate clinical service because restorations are exposed to different variables, such as temperature changes and constant humidity. This procedure reproduces, in a short period of time, the effects of long-term exposure of RBCs in an oral environment. However, as with most *in vitro* experiments, there are limitations to the accelerated aging process that do not consider clinical variables, such as the influence of the saliva components, pH levels, and brushing. Further, and as mentioned previously, bleaching treatments may affect the elution of monomers and other substances from RBCs, which may also affect the surface roughness of these materials and, as a consequence, changing color perception. A previous study showed changes in the value of L^* coordinate with different surface roughness values.[49]

After the accelerated aging process, L^* and WI_D values decreased (specimens became darker), while b^* values increased (specimens became more chromatic) for both composites (Table 3 through 6). In addition, a^* values generally increased. In all cases color changes and whiteness variations after aging procedures, which estimated 1 year and 2 years of clinical service,[21] were above acceptability thresholds: AT = 1.77 (Figure 2) and WAT = 2.90 (Figures 3 and 4). Therefore, the second part of the study hypothesis was accepted, meaning aging procedures produced color changes and whiteness variations of RBCs greater than the 50:50% acceptability thresholds (AT and WAT).

Previous studies also showed a decrease of L^* values and an increase of b^* values after artificial aging procedures of RBCs.[20,21,46-48] However, the assessment of color and/or whiteness variations in these studies was based solely on a quantitative evaluation without taking into account the limits of visual perception and, therefore, its clinical impact.

CONCLUSIONS

Within the limitations of this study, aging produced unacceptable color matches of RBCs while bleaching treatments produced, in most cases, imperceptible color changes of nanocomposites.

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Conflict of Interest

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

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Effects of pH and Application Technique of In-office Bleaching Gels on Hydrogen Peroxide Penetration into the Pulp Chamber

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Clinical Relevance

In-office bleaching gel with a neutral/alkaline pH resulted in less hydrogen peroxide penetration into the pulp chamber and could be safely applied using different techniques.

SUMMARY

Objective: This *in vitro* study aimed to quantify the penetration of hydrogen peroxide (HP) into the pulp chamber in teeth submitted to in-office bleaching with varied pH and application techniques. The color change and pH of the in-office bleaching product during application was also evaluated.

Methods and Materials: Ninety-six human premolars were used and randomly divided into 10 groups (n=9) according to the following combination of factors: pH of in-office bleaching agents (two neutral/alkaline pH: Opalescence Boost 38% and Whiteness HP Blue 35% and three acidic pH: Whiteness HP Maxx 35%, Lase Peroxide Sensy 35%, and Total Blanc Office 35%) and application modes (for 3 × 15 minutes [3×15] and 1 × 45 minutes [1×45]). An additional group of non-bleached teeth (control; n=6) was added. First, all teeth were sectioned 3 mm from the cementoenamel junction and the pulp tissue was removed. An acetate buffer was placed in the pulp chamber of all teeth. After bleaching, this solution was

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transferred to a glass tube in which HP was allowed to react with other components, resulting in a pink solution. The optical density of this pink solution was measured using ultraviolet-visible spectroscopy and converted into amount of HP. Color change before and 1 week after bleaching was evaluated using a digital spectrophotometer. A pH meter with a 6-mm circular and flat surface was used in contact with the enamel surface to quantify the pH of the bleaching gels during application. Data were analyzed using two-way analysis of variance and Tukey tests (α=0.05).

Results: Overall, lower mean HP penetration values were observed for Opalescence Boost 38% and Whiteness HP Blue 35% compared with other bleaching gels ($p<0.05$). Opalescence Boost 38% and Whiteness HP Blue 35% were not influenced by the application technique ($p>0.05$). However, lower mean HP penetration values were observed for Whiteness HP Maxx 35%, Total Blanc Office 35%, and Lase Peroxide Sensy 35% when using the 3×15 application technique compared with the 1×45 technique ($p<0.05$). Significant whitening was detected and no significant difference of color change was observed between groups ($p>0.54$). The pH did not change during the 3×15 application technique; however, all acidic bleaching gels significantly decreased in pH when applied for 1×45 ($p<0.01$).

Conclusions: The amount of HP that reaches the pulp chamber was lower when neutral/alkaline pH gels were used, independently of the application technique. When considering acidic pH gels, it is preferable to use the 3×15 application technique, mainly because longer application time (1×45) results in lower pH. No difference was observed between groups with regards to color change.

INTRODUCTION

Carbamide peroxide applied at home is the technique most employed by clinicians for bleaching vital teeth,[1,2] and it provides a high satisfaction rate among patients.[3] However, some patients have indicated that they could not adapt to the use of whitening trays or disliked waiting 2 to 3 weeks for the bleaching results.[4,5] In these cases, an in-office bleaching technique is indicated because it is possible to obtain faster bleaching results compared with the at-home treatment,[4,6] with the advantage of exclusive in-office application and full control of the whole procedure, which prevents the intake of product.[4,7,8]

Unfortunately, due to the higher concentration of hydrogen Peroxide (HP) used, in-office bleaching is related to a higher percentage of patients with tooth sensitivity (TS).[4-8] Bleaching-induced TS is associated with an inflammatory response from the pulp cells due to the diffusion of HP,[9] which may alter the dental pulp.[10,11] This is the main reason for several changes to the in-office bleaching gels recently launched by manufacturers.

In the past, in-office bleaching gels were delivered with a low pH (around 2.0)[12,13] to increase the product's shelf life.[14] However, this leads to several modifications in the chemical composition, morphology, and mechanical properties of the tooth structure,[15,16] which can increase the passage of HP and, consequently, increase TS.[17-20] These enamel alterations are more aggressive if the bleaching gels are in contact with the enamel surface for a long period of time. This occurs due to the prolonged contact of enamel with low pH in-office gels[21,22] and is one of the reasons manufacturers have indicated replenishing the gel during in-office bleaching. The HP is typically applied and left undisturbed on tooth surfaces for 10-20 minutes during in-office bleaching, and this procedure is repeated two to five times at each clinical appointment, depending on the bleaching gel brand.[23,24]

Recently, in-office bleaching gels began presenting with neutral to alkaline pH, which are less aggressive to the tooth structure.[21,22,25,26] Some clinical studies have shown that neutral/alkaline pH bleaching gels reduced TS compared with acidic pH bleaching gels.[17,19,20] Due to this, manufacturers have suggested that these new brands could be applied without replenishing the gel during in-office bleaching. This simplifies the clinical procedure, reduces the risk of occasional soft tissue burns, and lowers the cost because less material is used per patient.

Unfortunately, the pH of different available commercial products is not clear to clinicians, nor is the best application mode made known to clinicians. Therefore, the aim of the present study was to compare the amount of HP that reaches the pulp chamber using in-office bleaching gels with different pH applied using two application modes. Also, the pH of these in-office bleaching gels was evaluated during application, and color change was evaluated immediately and 7 days after bleaching.

Table 1: *Material, Batch Number, Composition, pH, and Manipulation for In-office Bleaching Gels*

Material/Batch Number	Composition	pH[a]	Manipulation[a]
Opalescence Boost PF 38% (190250)	38% Hydrogen peroxide, 20% water and desensitizing agents (3% potassium nitrate and 1.1% fluoride)	7.0	Attach both syringes before mixing. Press the plunger of the red syringe in, pushing all the contents into the clear syringe. Forcefully press the small clear stem completely into the larger clear stem. Then press the clear plunger completely into the red syringe. To activate, press the chemical from the red syringe into the clear syringe with thumbs. Reverse action, and mix a minimum of 25 times on each side
Whiteness HP Blue 35% (161012)	35% Hydrogen peroxide, deionized water, thickener, violet colorant, glycol, neutralized agents and desensitizing agents (3% calcium gluconate)	8-9	Prepare the gel: mix the two phases with the syringes connected, pushing the plungers four times on each side (total of eight times), then push all the mixed content into one of the syringes, and it is ready for use. Make sure both syringes are well attached; Place a tip on the syringe that still has the gel and apply a layer to the entire vestibular surface of the teeth to be whitened (including the interproximals) and extend a little onto the incisal and occlusal faces. The layer of gel should be between 0.5 and 1 mm thick.
Whiteness HP Maxx 35% (200814)	35% Hydrogen peroxide, thickener, colorants, glycol, filler, deionized water	6.5	Using the mixture plaque provided in the kit, mix the peroxide phase (phase 1) with the thickener phase (phase 2) in the following proportion: three drops of peroxide to one drop of thickener. Shake vigorously the thickener bottle before using it. This mix quantity is sufficient for one tooth application. With the help of a spatula, mix both phases for 10 seconds.
Lase Peroxide Sensy 35% (11002)	35% Hydrogen peroxide, thickener, water, vegetables extracts, sequestrating agents, amide, colorants and glycol	6.5	Remove the caps of the syringes with peroxide and thickener, and attach the syringes tightly. Make sure they are well connected to avoid material leakage. Poke the plungers alternately until the product reaches a homogeneous yellow coloring, indicating that it is active. Then, transfer all the mixture to the orange plunger syringe (hydrogen peroxide). Couple the applicator tip in the syringe and apply the bleaching gel over the teeth labial surface. The mixture content is sufficient for simultaneous application on the two arcades.
Total Blanc Office 35% (13040589)	35% Hydrogen peroxide, thickener, water, vegetable extracts, sequestrating agents, amide, colorants and glycol	6.5	Remove the caps of the syringes with peroxide and thickener and attach the syringes tightly. Make sure they are well connected to avoid material leakage. Poke the plungers alternately until the product reaches a homogeneous yellow coloring, indicating that it is active. Then, transfer all the mixture to the orange plunger syringe (hydrogen peroxide). Couple the applicator tip in the syringe and apply the bleaching gel over the teeth labial surface. The mixture content is sufficient for simultaneous application on the two arcades.

[a] *According to the manufacturer's indications.*

The null hypotheses tested were that the pH and application mode of in-office bleaching gels does not affect (1) the amount of HP that reaches the pulp chamber and (2) color change after bleaching.

METHODS AND MATERIALS

This study was reviewed and approved by the Ethics Committee of the local university, under protocol No. 1355037. A total of 96 sound premolar human teeth, free of fracture lines, enamel defects, fissures, or any pathologic lesions, were divided according to a combination of the following main factors: 1) bleaching agents (Opalescence Boost PF 38% [Ultradent, South Jordan, UT, USA]; Whiteness HP Blue 35% [FGM, Joinville, SC, Brazil]; Whiteness HP Maxx 35% [FGM]; Lase Peroxide Sensy 35% [DMC, São Carlos, SP, Brazil]; or Total Blanc Office 35% [Nova DFL, Estrada do Guerengué, RJ, Brazil]) (Table 1); and 2) application technique (three 15-minute applications [3×15] or a single 45-minute [1×45] application). An additional, unbleached control group was added to the experimental design.

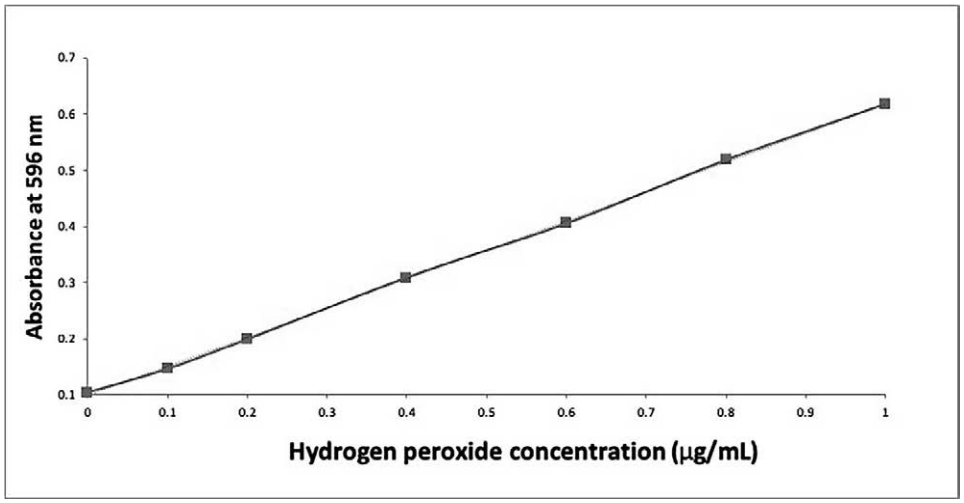

Figure 1. *Spectophotometric calibration curve used in this study (R=0.999524).*

Sample-Size Calculation

The primary outcome of this study was the amount of HP penetration inside the pulp chamber. According to previous literature, the amount of HP penetration for the control group was determined to be 0.6 ± 0.2 μg/mL.[27,28] Using an α of 0.05, a power of 80%, and a two-sided test, the minimal sample size was six teeth in each group in order to detect a difference of 0.3 μg/mL among the tested groups.

Quantification of HP Penetration

The roots of all teeth (n=6 for each group) were sectioned using a diamond disc (KG Sorensen, Barueri, SP, Brazil, double-sided segmented, No. 7011) under constant water irrigation 3 mm apical to the cementoenamel junction. The pulp tissue was then carefully removed, and the pulp chamber washed with distilled water. The pulp chamber was carefully widened with a round bur (#1014, KG Sorensen). The entrance to the pulp chamber was widened using a round bur (No. 1014, KG Sorensen) to accommodate a micropipette (LABMATE Soft, 50 μL of capacity, HTL Lab Solutions, Warsaw, Poland), while avoiding touching the pulp chamber walls.

All teeth were vertically fixed to a wax plaque. Adhesive tape (Missner, Missner & Missner Ltda, Blumenau, SC, Brazil) was placed before nail polish application to limit the exposure area to 6 mm (radius) (Colorama, L'Oreal Brasil, Rio de Janeiro, RJ, Brazil), standardizing the area for bleaching gel application and color measurement.

Before applying the bleaching gels, an analytic curve was prepared with a standard HP solution at 30% concentration (Labsynth, Diadema, SP, Brazil) to obtain the relation between the light absorbance and the HP concentration. For this purpose, an acetate buffer solution (pH 4.5) was used to attract and stabilize the HP that might penetrate the pulp chamber. The solution was titrated with a potassium permanganate standard solution (Figure 1).[29] Next, a 25-μL aliquot of acetate buffer (pH 4.5) was placed into the pulp chamber of each tooth to absorb and stabilize any HP that might penetrate the pulp chamber.

All bleaching gels were manipulated according to the description in Table 1 and placed over the enamel surface. In the 1×45 group, the product was placed on the enamel surface and remained untouched for the entire time (45 minutes). In contrast, the product on the tooth surface in the 3×15 group was removed using an aspirating tip, and the product reapplied two additional times, until 45 minutes had elapsed (Table 1).

After the treatment period, a micropipette was used to transfer the acetate buffer solution from the pulp chamber of each tooth to a glass tube. The pulp chamber of each tooth was then rinsed four times with 25 μL of acetate buffer, which was placed into the same glass tube. Then, deionized water (2.725 μL) was added to the glass tube along with 4 mmol/L of 4–amino-2,3-dimethyl-1-phenyl–3-pyrazolyl–5, 24 mol/L of phenol, 0.4 U/mL of peroxidase dissolved in phosphate buffer, 0.1 M pH = 7.0 (Glucose pp, Gold Análise Diagnóstica Ltda, Belo Horizonte, MG, Brazil). When stored at 4°C, the peroxidase catalyzes the degradation of HP in the presence of aminophenazone 4-aminoantipyrine with phenol. This reaction releases oxygen that oxidizes the chromogenic hydrogen donor, making the originally transparent solution turn to a shade of pink.

The amount of HP in the solution was then measured by evaluating the color absorbance using an ultraviolet-Vis spectrophotometer (Shimadzu UV 1601, Kyoto, Japan) at a wavelength of 510 nm to obtain the optical density.[30] According to Beer's Law, absorbance is directly proportional to the concentration; therefore, the concentration of HP (μg/mL) was determined by comparing it to the calibration curve obtained previously (Figure 1).[29,30] Once the concentration (μg/mL) and volume of the solution were known, the HP mass (l g) was calculated using the following equation: m = C * MM * V, where m represents mass, C is the concentration, MM is the HP molar mass (34.158), and V is the volume (3×10^{-3} L).[30] This procedure was repeated separately for each tooth.

Color Change Evaluation

The same teeth used in the "Quantification of HP Penetration" section were used for color change measurement. The color change was evaluated using the spectrophotometer, VITA Easyshade (VITA Zahnfabrik, Bad Säckingen, Germany). The tip of the device was put in contact with the previously delimited area and the L*, a*, and b* parameters of color were obtained from the spectrophotometer. The L* value represents the luminosity (value from 0 [black] to 100 [white]), the a* value represents the measurement along the red-green axis, and the b* value represents the measurement along the yellow-blue axis. The color change (ΔE) before (baseline) and 1 week after the bleaching procedure was given by differences between the two colors measured with the spectrophotometer, which was calculated using the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. All teeth were immersed in distilled water at 37°C from the completion of the bleaching procedures until the final color was measured.

Quantification of pH in Contact With Enamel Surface

Thirty sound premolar human teeth were used in this part of the study (n=3 for each group). All teeth were fixed vertically to a wax plaque, and adhesive tape (Missner, Missner & Missner Ltda) was placed before nail polish application to limit the exposure area to 5 mm^2 (Colorama, L'Oreal Brasil) to standardize the area of bleaching gel application. All bleaching gels were manipulated according to the description in Table 1 and placed over the enamel surface as described previously.

A pH meter with a 6-mm circular and flat surface pH electrode (Extech pH100: ExStik pH Meter; Extech instruments, Nashua, NH, USA) was positioned directly onto the delimited area and held in position until the pH was stabilized.[20] Because the pH electrode is very sensitive, it was possible to make three measurements for each tooth. For both application protocols (3×15 and 1×45 applications), pH was registered at the baseline and every 15 minutes until 45 minutes had elapsed.

Table 2: *Means and Standard Deviations of the Hydrogen Peroxide (HP) Concentration (μg/mL) Detected Inside the Pulp Chamber for the Treatment Groups and the Statistical Comparison*

Bleaching Gel	Application Technique[a]	
	3×15 min	1×45 min
Opalescence Boost PF 38%	0.13 ± 0.06 A	0.19 ± 0.04 A,B
Whiteness HP Blue 35%	0.12 ± 0.02 A	0.19 ± 0.08 A,B
Whiteness HP Maxx 35%	0.14 ± 0.08 A	0.32 ± 0.07 C
Lase Peroxide Sensy 35%	0.19 ± 0.08 A,B	0.28 ± 0.04 C
Total Blanc Office 35%	0.21 ± 0.06 A,B	0.30 ± 0.10 C
Control	0.03 ± 0.002*	

[a] *Different letters indicate that groups are statistically different (two-way analysis of variance, Tukey test, p<0.05).*
** All groups showed higher and statistically significant values compared with the control group (Dunnett test, p=0.00001)*

Statistical Analysis

The data from the HP concentrations and ΔE were subjected to a two-way analysis of variance (ANOVA) (bleaching agent and application technique) and Tukey test for pairwise comparisons. Also, all groups from the HP concentrations were compared against the control group using the Dunnett test. The data from the pH values were compared using a two-way repeated measures ANOVA (bleaching agent and application technique). All tests were carried out using the Sigma Plot 11 software (Systat Software Inc, Chicago, IL, USA) with a preset level of significance of 5%.

RESULTS

Quantification of HP Penetration

The HP concentration that reached the pulp chamber in each group is depicted in Table 2. The two-way ANOVA revealed a statistically significant effect for the cross-product interaction (Table 2; $p=0.001$). The amount of HP was lower in the pulp chamber of the control group than the bleached groups (Table 2; $p<0.05$). In general, there was no difference between the in-office bleaching agents tested when they were used in the 3×15 technique (Table 2; $p>0.41$). However, there was a significant difference when the gels were applied using the 1×45 technique

Table 3: *Means and Standard Deviations of the ΔE for the Treatment Groups and the Statistical Comparison*

Bleaching Gel	Application Techniques[a]	
	3×15 min	1×45 min
Opalescence Boost PF 38%	13.2 ± 3.3	14.2 ± 3.4
Whiteness HP Blue 35%	12.7 ± 2.9	13.7 ± 3.2
Whiteness HP Maxx 35%	12.3 ± 2.9	12.7 ± 3.9
Lase Peroxide Sensy 35%	11.7 ± 4.5	12.5 ± 4.1
Total Blanc Office 35%	12.2 ± 3.2	12.1 ± 3.9

[a] *No significant differences were observed between different groups (two-way analysis of variance, Tukey test, p>0.54).*

(Table 2; $p=0.001$). In the 1×45 technique, the neutral/alkaline in-office bleaching gels Opalescence Boost PF 38% and Whiteness HP Blue 35% showed lower mean HP penetration than the acidic bleaching gels (Table 2; $p=0.001$).

Color Change Evaluation

The ΔE in each group is shown in Table 3. Two-way ANOVA revealed no statistically significant effect for the cross-product interaction and the main factors (Table 3; $p>0.54$). These results indicate that no significant difference in color change was observed between groups. However, a significant whitening effect was detected by all groups, regardless of the pH and application mode of the evaluated in-office bleaching gels (Table 3).

Quantification of pH in Contact With Enamel Surface

The quantification of pH in contact with the enamel surface in each group is shown in Table 4. The two-way ANOVA revealed a statistically significant effect for the cross-product interaction (Table 4; $p=0.01$). The baseline results confirmed that Opalescence Boost PF 38% and Whiteness HP Blue 35% are neutral/alkaline in-office bleaching gels. On the other hand, Whiteness HP Maxx 35%, Lase Peroxide Sensy 35%, and Total Blanc Office 35% were confirmed to be acidic in-office bleaching gels (Table 4). The pH remained stable during the 3×15 application technique for all bleaching gels evaluated (Table 4; $p>0.05$). However, when applied in the 1×45 technique, there was a significant difference between the in-office bleaching agents tested (Table 4; $p=0.01$). When the neutral/alkaline in-office bleaching gels were applied, no significant change in pH was observed during the 1×45 application (Table 4; $p>0.61$). On the other hand, all acidic bleaching gels significantly decreased in pH during the 1×45 technique, mainly after 30 minutes (Table 4; $p<0.01$).

DISCUSSION

The results of the present study showed that when the different application techniques were evaluated, a significant difference was observed for the acidic pH in-office gels compared with the other gels, leading us to reject the first null hypothesis. A higher amount of HP was usually found inside the pulp chamber for the 1×45 technique. According to the pH measurement, three products (Whiteness HP Maxx 35%, Lase Peroxide Sensy 35% and Total Blanc Office 35%) were considered acidic pH in-office gels in agreement with the measurements of several authors.[21,22,26] This is expected because acidic gel could cause more microhardness loss and morphologic change of enamel due to the enamel surface demineralization compared with the neutral/alkaline gels.[15,16]

The results of the present study also showed a significant decrease in the pH of the acidic in-office gels with prolonged contact with enamel.[21,22] This may lead to further damage to the surface of the teeth, resulting in an increase in the superficial porosities of the enamel[15,16,31] and, consequently, greater passage from HP to the pulp chamber, in agreement with the results of HP penetration in the present study. This is the main reason the replen-

Table 4: *Means and Standard Deviations of pH at the Different Assessment Points for the Treatment Groups and the Statistical Comparison*

Bleaching Gel	3×15 Application Technique[a]				1×45 application technique[a]			
	Baseline	15 min	30 min	45 min	Baseline	15 min	30 min	45 min
Opalescence Boost PF 38%	7.5 ± 0.3 A	7.5 ± 0.6 A	7.6 ± 0.3 A	7.8 ± 0.3 A	7.4 ± 0.3 A	7.5 ± 0.6 A	7.7 ± 0.4 A	7.7 ± 0.4 A
Whiteness HP Blue 35%	7.7 ± 0.4 A	7.6 ± 0.2 A	7.9 ± 0.2 A	8.0 ± 0.2 A	7.8 ± 0.3 A	7.5 ± 0.3 A	7.7 ± 0.3 A	7.4 ± 0.3 A
Whiteness HP Maxx 35%	6.5 ± 0.3 B	6.4 ± 0.3 B	6.6 ± 0.2 B	6.6 ± 0.4 B	6.5 ± 0.4 B	6.2 ± 0.4 B	5.7 ± 0.2 B,C	5.3 ± 0.3 C
Lase Peroxide Sensy 35%	6.1 ± 0.2 B	6.1 ± 0.3 B	6.2 ± 0.4 B	6.3 ± 0.2 B	6.1 ± 0.3 B	5.7 ± 0.2 B,C	5.5 ± 0.3 B,C	5.0 ± 0.3 C
Total Blanc Office 35%	6.4 ± 0.5 B	6.3 ± 0.4 B	6.2 ± 0.3 B	6.4 ± 0.3 B	6.3 ± 0.2 B	5.8 ± 0.3 B	5.6 ± 0.3 B,C	5.2 ± 0.4 C

[a] *Different letters indicate that groups are statistically different (two-way repeated measures analysis of variance, p=0.01).*

ishing technique is indicated for the majority of in-office bleaching gels available on the market.[7,32] When the acidic gel is removed after 15-20 minutes of application, it prevents further changes on the enamel surface and, consequently, decreases the passage of HP, as observed in the results of the present study.

On the other hand, the results of the present study showed no significant difference for Opalescence Boost PF 38% and Whiteness HP Blue 35% with regards to the application technique. According to the pH measurement, these gels are considered neutral/alkaline pH gels. It has been described that a higher pH in bleaching gels leads to more dissociation of HP into free radicals. For instance, HP with a pH of 9 dissociated 2.7 times more than HP with a pH of 4.4.[33] Thus, if more HP dissociates into free radicals within the dental structure, less surplus of HP is available to travel within the dentin and reach the pulp chamber. This may explain the lower amount of diffused HP for the Opalescence Boost PF 38% and Whiteness HP Blue 35%.

When the 1×45 technique was evaluated, the neutral/alkaline gels typically showed a lower amount of HP inside the pulp chamber compared with the acidic gels, which was previously shown by Mena-Serrano and others.[27] This is in agreement with recent published clinical studies that showed a lower percentage of patients who reported TS when neutral/alkaline pH bleaching gels were compared with acidic pH bleaching gels.[17,19,20]

The results of the present study and the study of Mena-Serrano and others[27] are not in consensus with the current literature. For instance, Pignoly and others[34] and Marson and others[35] showed that the amounts of HP found in the pulp chamber were similar, regardless of the pH of the gel used. However, methodologic differences may explain this controversy. While some studies used a small part of a bovine tooth, turning it into a simulated pulp chamber, the present study used human teeth without any preparation, which better simulates the clinical situation.[34,35] Also, Kwon,[36] who was the first to introduce the protocol of not replenishing the gel during in-office bleaching more than 10 years ago, showed that the amount of HP inside the pulp chamber was significantly lower when one prolonged application was evaluated. However, the application of the bleaching gel was evaluated using a different technique (sealed technique), so a clear comparison cannot be made between the results of the present study and Kwon's results.[36]

It is worth mentioning that the results of the present study showed that these neutral/alkaline pH gels maintain their pH for the entire application duration, which was also previously observed by Trentino and others.[21] This could be explained by the fact that these gels have a pH that is almost neutral; few ion exchange reactions occur with the surface of the enamel,[37,38] which enables pH to be maintained. This helps to explain why the application technique did not significantly change the amount of HP inside the pulp chamber when neutral/alkaline gels were applied.

Unfortunately, the products with the lower concentration of HP in the pulp chamber (alkaline/neutral gels) contain desensitizing agents. Opalescence Boost PF 38% contains potassium nitrate and sodium fluoride, and Whiteness HP Blue 35% contains calcium digluconate. These agents act in different ways; for instance, potassium nitrate reduces sensitivity by decreasing the capacity of the nerve fibers to propagate nerve impulses. On the other hand, sodium fluoride and calcium digluconate act by blocking the dentinal tubules, leading to a reduced flow of fluid into the pulp chamber.[39] A closer view of clinical trials that evaluated the TS of in-office bleaching gels containing desensitizer agents have shown controversial results.[17,19,20,40] Future clinical studies need to be done to evaluate the effect of neutral/alkaline gels with and without desensitizer on HP penetration.

It is also worth mentioning that it is not only the pH and the addition of desensitizing agents that could influence the amount of HP inside the pulp chamber. Other factors may affect the amount of HP that enters the pulp cavity, such as HP concentration and the viscosity of the bleaching gels.[27,41] Future studies need to be done to evaluate how these factors interact with the pH of different bleaching gels.

Finally, independent of the pH and application technique applied, a significant whitening was detected and no significant difference in color change was observed between groups, leading us to accept the second null hypothesis. This is in agreement with several clinical studies that have shown that all in-office bleaching materials evaluated in the present study are effective in terms of whitening.[17-19,20,40] However, according to the results of the present study, the pH and application technique of in-office bleaching gels could impair the amount of HP reaching the pulp chamber.

CONCLUSION

The amount of HP that reaches the pulp chamber was lower when neutral/alkaline pH gels were used, independent of the application technique. For acidic pH gels, it is preferable to use the 3×15 application technique, mainly because the 1×45 technique results in lower pH.

Acknowledgements

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of State University of Ponta Grossa. The approval code for this study is 1355037.

Conflict of Interest

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

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Online Only Articles

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Letter to the Editor re: Prospective Clinical Study of Zirconia Full-coverage Restorations on Teeth Prepared With Biologically Oriented Preparation Technique on Gingival Health: Results After Two-year Follow-up

JL D'Souza • M Kundabala • N Shetty

AUTHOR RESPONSE

Streptococcus mutans Biofilm Formation and Cell Viability on Polymer-infiltrated Ceramic and Yttria-stabilized Polycrystalline Zirconium Dioxide Ceramic

MA Bottino • SMB Pereira • M Amaral • NVM Milhan • CA Pereira SEA Camargo • ABG Carvalho • RM Melo

Clinical Relevance: Y-TZP ceramics are bioinert materials and present good biocompatibility to oral tissues. A polymer-infiltrated ceramic, which is a hybrid material, presents the same biological characteristics as Y-TZP ceramics.

https://doi.org/10.2341/18-278-L

Diagnostic Value of the Basic Erosive Wear Examination for the Assessment of Dental Erosion on Patients, Dental Photographs, and Dental Casts

T Wohlrab • S Flechtenmacher • J Krisam • D Saure • D Wolff • C Frese

Clinical Relevance: The assessment of the basic erosive wear examination (BEWE) on patients and on dental photographs seems to yield comparable results and therefore may be a suitable tool for longitudinal monitoring of dental erosion. The assessment of BEWE on dental casts may better be used for laboratory techniques.

https://doi.org/10.2341/18-127-C

Effect of Immediate Dentin Sealing and Surface Conditioning on the Microtensile Bond Strength of Resin-Based Composite to Dentin

CRG van den Breemer • M Özcan • MS Cune • AP Almeida Ayres • B Van Meerbeek • MMM Gresnigt

Clinical Relevance: For partial indirect restorations, immediate dentin sealing is recommended, as bond strength remains stable over time.

https://doi.org/10.2341/18-052-L

Effect of Ferrule Thickness on Fracture Resistance of Teeth Restored With a Glass Fiber Post or Cast Post

PE Fontana • TC Bohrer • VF Wandscher • LF Valandro • IF Limberger • OB Kaizer

Clinical Relevance: A tooth without a ferrule presented more favorable failures than with a 1-mm-thick ferrule when restored with a cast post and core, despite an increased fracture resistance. The findings support the use of a glass fiber post.

https://doi.org/10.2341/18-241-L

Letter to the Editor

Dear Editor,

We have read the article titled "Prospective Clinical Study of Zirconia Full-Coverage Restorations on Teeth Prepared With Biologically Oriented Preparation Technique on Gingival Health: Results After Two-Year Follow-up" in September/October 2018 (Vol. 43, Issue 5).[1] We appreciate the authors for their novel approach for previously failed restorations, meticulous explanation of the technique, and two-year follow-up of cases. The described technique is beneficial for us since we come across many cases with similar problems.

We have a few doubts regarding the technique that we would like the author to clarify. According to Ivkovic and others, autopolymerized acrylic produces more cytotoxicity because of monomer leaching out, depending on internal and external factors. This could hinder the healing the gingiva.[2] Subgingival restorative margin placement has demonstrated adverse inflammatory periodontal reaction due to the tooth-restoration interface being overcontoured, difficulty in finishing and polishing of restorative margins, challenges in applying oral hygiene measures, increased pathogenicity of the subgingival dental plaque, and violation of the biologic width.

Moreover, according to Gianluca Paniz and others, feather edge preparation of margins presents significantly more bleeding on probing than chamfer preparation. They studied the periodontal response to two different subgingival restorative margin designs where follow-up for 12 months concluded that significant differences were seen in regard to plaque index, gingival index, and periodontal probing depth, but there was no statistically significant difference between chamfer and feather edge finishing lines in regard to these parameters.[3] According to Schatzle and others, after 26 years of follow-up, full-coverage crowns with subgingivally placed finishing lines had a detrimental effect on periodontal health. They also found deterioration of the clinical periodontal parameters within one to three years after the delivery of the restorations.[4]

With all the background above, we would like authors to give their opinion regarding the outcome of the subgingival placement of knife-edge margins that they have described. We once again thank the authors and *Operative Dentistry* for publishing this eye-opening article.

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Author Response

Thank you for your interest in our article. It is important to us that our work generates positive expectations, and in the case of the biologically oriented preparation technique (BOPT), it is well deserved, as the clinical results have been really spectacular.

Below are our responses to your questions:

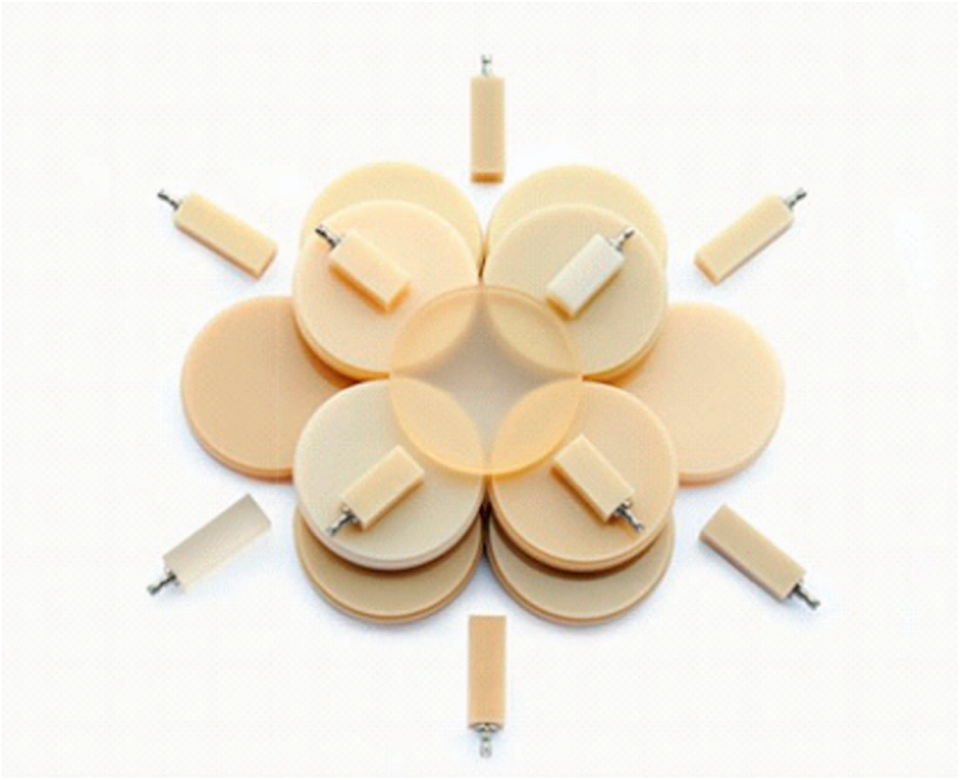

Figure 1. *Sintodent resin products for use with CAD/CAM methodology. Figure retrieved from http://www.sintodent.it on 1/Oct/19. Used by permission. http://www.sintodent.it/images/download/citotos-en.pdf; http://www.sintodent.it/images/download/certqualdisc-en.pdf).*

According to Ivkovic and others, autopolymerized acrylic produces more cytotoxicity because of monomer leaching out, depending on internal and external factors. This could hinder the healing the gingiva.

Reply: We are aware of what has been published regarding generalized monomer release deriving from self-polymerizing resins. However, we minimized the exposure to monomers by using a specific, carefully chosen resin: Sintodent (Sintodent S.r.l., Rome, Italy). This allowed us to design the provisional restoration digitally before dental preparation (CAD), which was milled in a five-axis milling machine (CAM) (Figure 1).

As soon as the tooth was prepared, the provisional restoration was relined with resin of the same composition but using a powder-liquid mixture. This particular acrylic resin has been investigated in several studies[1,2] that showed that its behavior is different from other acrylic resins, as it presents low contraction, a reduced exothermic phase, great strength, easy polishability, and a very important bacteriostatic function during the gingival healing phase.

In addition, once cured, we placed the resin in a high-temperature high-pressure kiln to achieve optimal polymerization, minimizing monomer release to avoid producing irritation or mucosa maceration. Afterward, we applied a layer of photopolymerizable resin nanofiller (GC Optiglaze varnish 15 ml, GC Corp, Tokyo, Japan) in the area of contact between the provisional restoration and tissue to isolate it from any released monomer and so improve the periodontal healing process. In addition, we used a light-curing elastomeric resin provisional cement, making it possible to remove excess cement *en bloc* (TempBond Clear, Kerr Corp, Orange, CA, USA).

Subgingival restorative margin placement has demonstrated adverse inflammatory periodontal reaction due to the tooth-restoration interface being overcontoured, difficulty in finishing and polishing of restorative margins, challenges in applying oral hygiene measures, increased pathogenicity of the subgingival dental plaque, and violation of the biologic width.

Reply: With BOPT, overcontouring is entirely different from what constitutes cervical overcontouring over a horizontal finishing line. We must distinguish between what is defined as the anatomical crown and what is described as a tooth's clinical crown. In BOPT, we modify the convexity of the anatomical crown so that the prosthesis imitates the

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http://www.sintodent.it/images/grafici/graficoattaccobatterico-it.pdf
as of 1/Oct/2019.

Figure 2. *Lifetime in contact with Sintodent resin of the most frequent microorganisms in the oral cavity. Ceded image of the manuscript: Albergo G, Sampalmieri F, Mattioli Belmonte M, Furore G, & Andreana S (2003) Attività antimicrobica di una resina acrilica [Antimicrobial activity of an acrylic resin]* Dental Cadmos ***71(2)*** *69-74.*

natural tooth, on which we have previously eliminated any horizontal-convex component that may present above the cemento-enamel junction (CEJ). But, with a horizontal finishing line, the emergence of the tooth's clinical crown is modified, which is where the well-known periodontal problems described in the literature arise, as this favors the accumulation of dental plaque resulting from aberrant anatomy. It must be understood that with BOPT, we imitate the convex anatomy of the natural tooth above its CEJ.

Moreover, according to Gianluca Paniz and others, feather edge preparation of margins presents significantly more bleeding on probing than chamfer preparation. They studied the periodontal response to two different subgingival restorative margin designs where follow-up for 12 months concluded that significant differences were seen in regard to plaque index, gingival index, and periodontal probing depth, but there was no statistically significant difference between chamfer and feather edge finishing lines in regard to these parameters.

Reply: We agree that Paniz and others in their articles after six months[3] and 12 months[4] found that there was gingival stability but slight periodontal inflammation around teeth prepared with BOPT. While these articles appear convincing, the BOPT protocol is not clearly defined. BOPT is very method and skill dependent and involves a learning curve of at least a year. We cannot be certain, but some results reported in these articles may be due to the following: 1) Methodological bias: the clinical protocol applied is not well defined in the article; randomization of the patient sample is not reported either (the patient-dependent periodontal variables of each subject conforming the sample are questionable), and 2) only one year follow-up is insufficient to assess clinical responses to a treatment.

The most important variable to consider in order to achieve a good outcome using BOPT is correct diagnosis of the tooth to be treated. The tooth must be free of active periodontal disease with a good prognosis for restoration. It is also important to carry out the right clinical-prosthetic protocol, as this technique is susceptible to iatrogenic damage through unmanaged invasion of the biological sulcus (Figure 3). BOPT must be the right choice for the case, and the clinician must be well trained to carry out dental preparation correctly. The fabrication of the provisional must be correct too; the dental technician must be instructed correctly so that the definitive prosthesis matches the biological parameters stipulated by the clinician in the provisional (Figure 4).[5,6]

According to Schatzle and others, after 26 years of follow-up, full-coverage crowns with subgingivally placed finishing lines had a detrimental effect on periodontal health. They also found deterioration of the clinical periodontal parameters within one to three years after the delivery of the restorations.

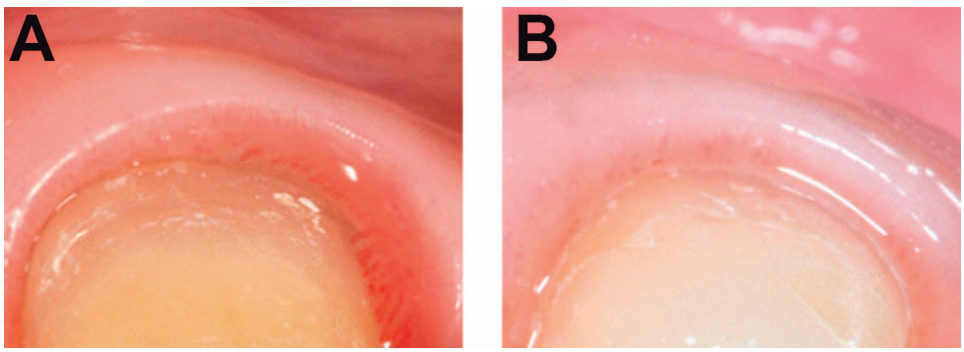

Figure 3. *(A, B): Healed gingival tissue after periodontal maturation around prosthesis emergence on tooth prepared with BOPT.*

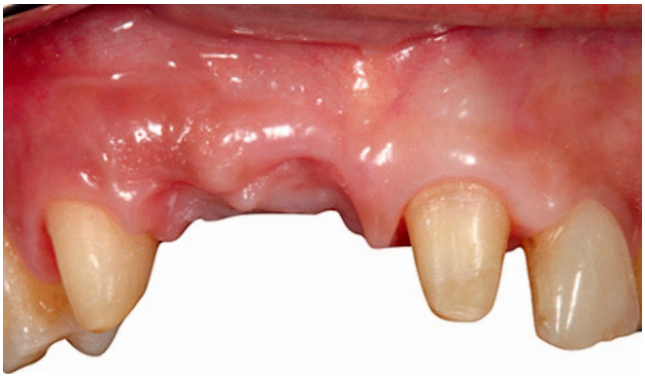

Figure 4. *Stabilization of gingival tissue eight weeks after treatment with BOPT.*

Reply: It is important to understand that BOPT differs from knife-edge preparation, as it creates a vertical plane with contouring and a prosthetic emergence angle that imitates the anatomical crown of a natural tooth (the angulation of this emergence does not have to be the same as with knife edge, which can reach a maximum of 90°) (Figure 5).[6]

We can classify dental preparation techniques for full-coverage crowns as two types: with or without a finishing line. In cases where it is decided to create a finishing line, preparation can have a sliding-vertical line (knife edge) or a horizontal finishing line (curved or flat chamfer, straight shoulder, 120° shoulder, beveled shoulder, and so on). With these types of preparation, the tooth-prosthesis interface may be positioned at different apico-coronal levels in relation to the gingival margin (supragingival, juxtagingival, and subgingival). The other option is using no dental finishing line, known as BOPT, first described by Dr Ignazio Loi in 2013.[7-10] With this technique, the tooth-prosthesis interface is always placed subgingivally (managed invasion of the periodontal sulcus).

Gingival placement of the preparation margin in indirect restorations has always been a topic of debate among dental professionals. Some researchers defend placement of the margin away from the epithelial insertion of the periodontum (juxta- or supragingival) in order to eliminate any factor that might cause gingival inflammation. Others have not found significant differences derived from gingival placement of the margin. There are cases in which the dentist has no other option than to position the preparation margin inside the periodontal sulcus, for example, in cases of subgingival oblique fracture, the presence of radicular caries, a tooth stump of dark color, sensitivity, cervical abrasion, or insufficient retention due to a short dental post.[11-17]

BOPT eliminates—by means of dental milling with diamond burs—the emergence of the anatomical crown above the CEJ, making it possible to fabricate a restoration with a new anatomical crown that respects periodontal tissue, facilitating periodontal tissue stabilization around the cervical area.[10]

Recent studies, case series, and prospective studies (with up to four years of follow-up)[6] vouch for the positive periodontal behavior around teeth prepared with BOPT, which present healthy and stable pericoronal tissue. The clinical advantages of BOPT are the following: 1) it eliminates the CEJ of the tooth, creating a new junction with the cervical margin of the indirect restoration (prosthetic CEJ); 2) it is possible to position the restoration's cervical margin at different levels inside the gingival sulcus without affecting the marginal fit between the dental preparation and the restoration (restoration over-

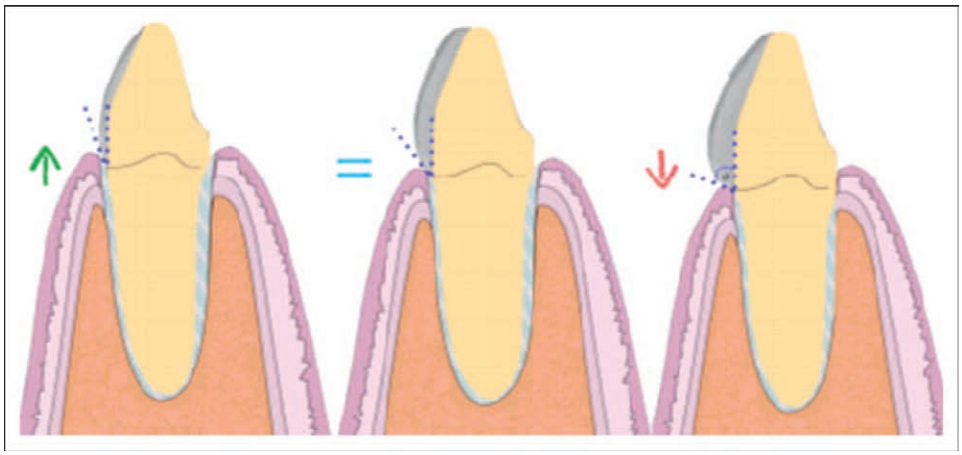

Figure 5. *Modification of the gingiva with respect to the prosthetic emergency. Ceded image of the manuscript: Agustín-Panadero R, Ausina-Escrihuela D, Fernández-Estevan L, Román-Rodríguez JL, Faus-López J, Solá-Ruíz MF (2017) Dental-gingival remodeling with BOPT no-prep veneers* Journal of Clinical and Experimental Dentistry ***9(12)*** *1496-1500. Figure used by permission.*

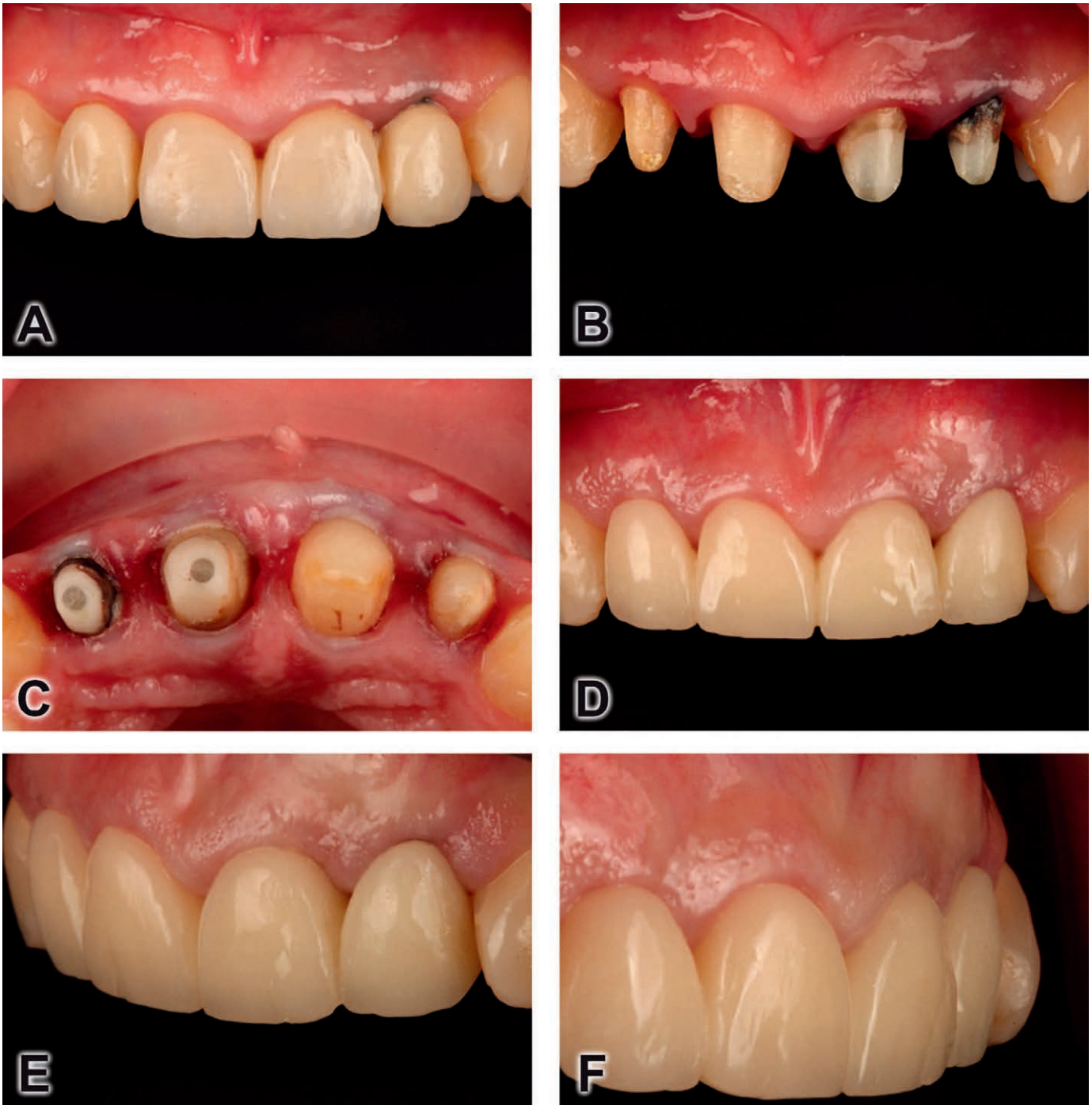

Figure 6. *Modification of the gingival emergence profile and periodontal health 12 weeks after treatment with vertical dental preparation. (A, B, C): Initial situation of the gingiva and old prosthesis in the anterior sector. (D, E, F): Soft tissue management with the provisional prosthesis. (G, H): Gingiva healed after BOPT treatment. (I, J): Final situation with the BOPT prosthesis.*

contouring); 3) it is possible to displace the gingival margin in an apico-coronal direction, modifying the convexity of the restoration's cervical area; and 4) the gingival margin is stabilized, and gingival thickness is increased.

On the other hand, the disadvantages of BOPT are the following: 1) it is a more complex technique that requires more clinical time and a learning curve, 2) situating the restoration margin in the right position is difficult given that there is no finishing line (risk

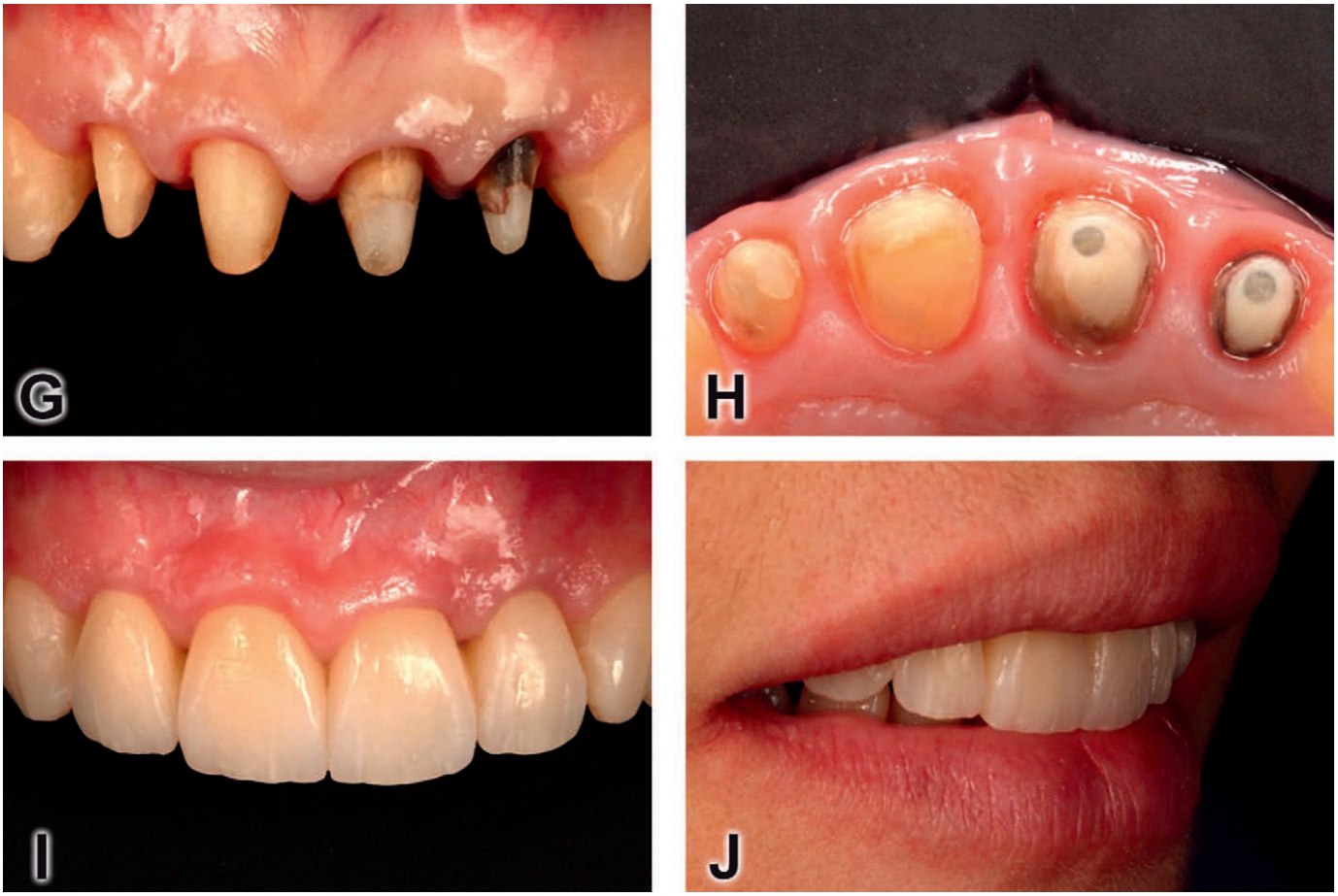

Figure 6. *Modification of the gingival emergence profile and periodontal health 12 weeks after treatment with vertical dental preparation. (A, B, C): Initial situation of the gingiva and old prosthesis in the anterior sector. (D, E, F): Soft tissue management with the provisional prosthesis. (G, H): Gingiva healed after BOPT treatment. (I, J): Final situation with the BOPT prosthesis. (cont.)*

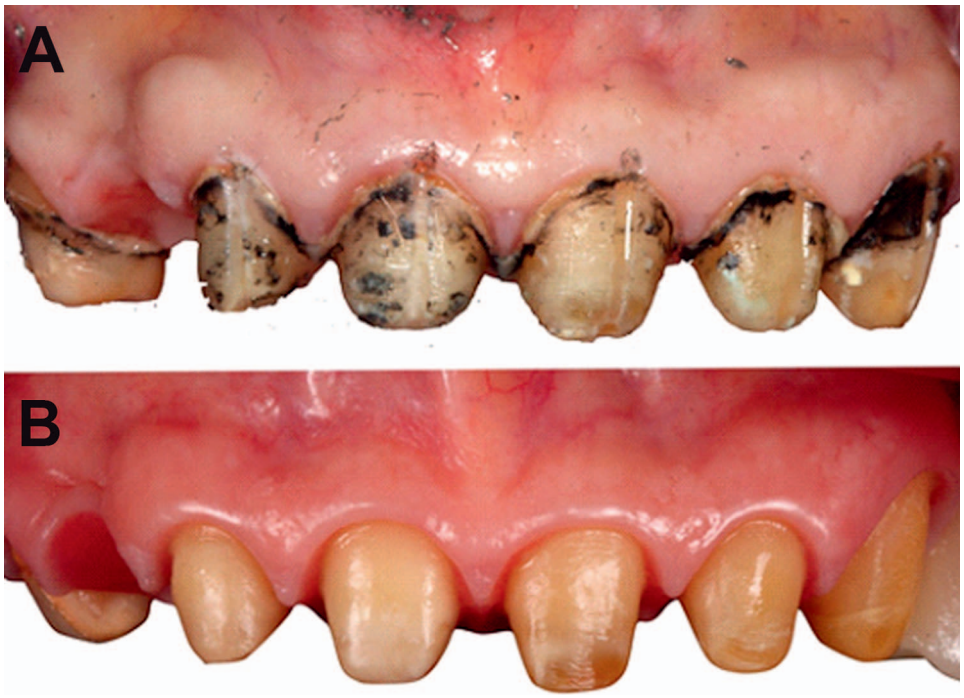

Figure 7. *(A, B): Gingival emergence anatomy after retreatment with BOPT*

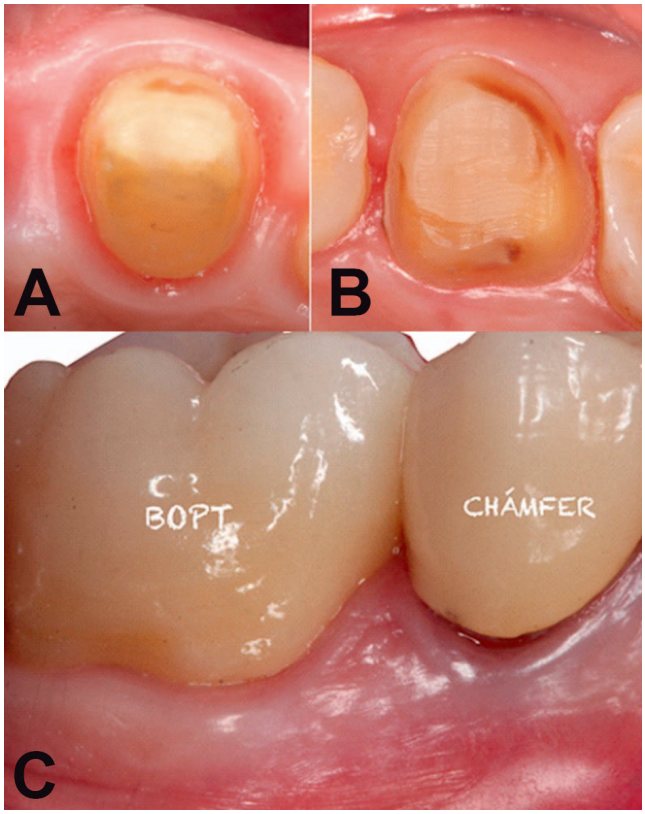

Figure 8. *(A, B, C): Gingival differences between restorations with and without dental finishing lines.*

of iatrogeny), and 3) removing excess cement when the dental preparation-restoration interface is positioned subgingivally is difficult (Figure 6).[7]

BOPT consists of milling the tooth to create a vertical axial plane between the dental anatomical crown and the apical area. The tooth reduced using BOPT has no dental finishing line (not knife edge), as this exists only on the prosthetic restoration and is characterized by cervical contouring determined in relation to the periodontal parameters of the tooth being restored (generating gingival margin stability) (Figure 7). Although BOPT has come into use only a short time ago, the literature published to date reports promising results in the medium term—a cause for optimism. These articles provide evidence of good clinical behavior, gingival marginal stability, and increased gingival thickness around the prosthetic emergence—all aspects of particular concern to restorative dentists (Figure 8).[5-10]

Many thanks for your interest in our article. We hope we have provided adequate responses to your queries. Below is an up-to-date bibliography for BOPT and the periodontal behavior of teeth prepared and restored with a finishing line.

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Dear R Agustín-Panadero, B Serra-Pastor, A Fons-Font, & MF Solá-Ruíz:

First of all, we would like to thank the authors for their prompt reply and detailed description of the work done. We appreciate the meticulous work and research you have done regarding the BOPT technique. The material used for the fabrication of provisional restoration and cementation along with its properties, which do not hinder the gingival healing, gives a clear idea of the differences from conventional acrylic. The explanation given on how the BOPT technique differs from various different finish lines and the advantage over others convinces us that this technique could be incorporated into our clinical practice. Even though the procedure is a more complex technique that requires more clinical time and a learning curve, it can be practiced because of the long-term success of this procedure along with maintaining good periodontal health, which you have shown in your cases. Thank you very much for sharing the information.

JL D'Souza, M Kundabala, N Shetty

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Streptococcus mutans Biofilm Formation and Cell Viability on Polymer-infiltrated Ceramic and Yttria-stabilized Polycrystalline Zirconium Dioxide Ceramic

MA Bottino • SMB Pereira • M Amaral • NVM Milhan
CA Pereira • SEA Camargo • ABG Carvalho • RM Melo

Clinical Relevance

Y-TZP ceramics are bioinert materials and present good biocompatibility to oral tissues. A polymer-infiltrated ceramic, which is a hybrid material, presents the same biological characteristics as Y-TZP ceramics.

SUMMARY

Objective: The aim of this study was to investigate the biofilm formation and cell viability of a polymer-infiltrated ceramic (PIC) and an yttria-stabilized polycrystalline zirconium dioxide ceramic (Y-TZP). The null hypothesis was that there would be no difference in biofilm formation and cell viability between the materials.

Methods and Materials: *Streptococcus mutans* biofilm was analyzed with scanning electron microscopy (SEM), confocal laser scanning microscopy, and colony counting (colony-forming units/mL). The cell viability (fibroblasts) of

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both materials was measured with 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium) (MTT) test. Roughness measurements were also performed.

Results: The PIC displayed higher roughness but showed similar colony-forming units and biovolume values to those of Y-TZP. SEM showed a higher amount of adhered fibroblasts on the PIC surface on the first day and similar amounts on both materials after seven days. Moreover, the materials were biocompatible with human fibroblasts.

Conclusion: PIC and Y-TZP are biocompatible and present the same characteristics for biofilm formation; therefore, they are indicated for indirect restorations and implant abutments.

INTRODUCTION

Ideally, lost dental structure should be replaced with materials with properties as close as possible to the natural tissues.[1] Currently, dental ceramics and composites are the materials most employed for restorations in the oral environment.[2,3] Therefore, the microstructure and the surface and mechanical properties, in addition to the interactions of such materials with the oral environment, as well as the capacity of retaining biofilm should all be well known before these materials are used in clinical practice.

A hybrid composite (Vita Enamic, Vita Zahnfabrik, Bad Säckingen, Germany) with about 14% of polymer distributed in the ceramic matrix has recently entered the dental restoration market. According to the manufacturer, this material combines strength with elasticity, allowing its use as implant abutments, where only zirconia was previously indicated. On the other hand, the well-known zirconium oxide–based ceramic (yttria-stabilized polycrystalline zirconium dioxide ceramic [Y-TZP]) is a polymorphous crystal stabilized by oxides (usually yttrium oxide) in the tetragonal phase at room temperature, with high strength and elastic modulus.[4] Both zirconia and hybrid material present optimal esthetic and mechanical properties for dental restorations.[1,2,5,6] However, little is known about the polymer-infiltrated material with regard to bacterial adhesion (ie, biofilm formation) and cell compatibility.

Biofilm formation may lead to several negative biological responses in the oral environment,[7] such as peri-implantitis,[8] injury to gingival tissues when colonization is located at the interface of a restoration at the gingival margin,[9] and secondary caries and pulp pathologies when it invades the restoration-tooth interface.[10,11] Biofilm formation on the surface of restorative materials may be evaluated semiquantitatively by counting colony-forming units (CFU)[12,13] and confocal laser scanning microscopes (CLSM),[14,15] as well as qualitatively by scanning electron microscopes (SEMs).[16]

Overall, ceramics are noncytotoxic, but several of them decrease cell proliferation after aging.[17] Therefore, the dual composition of newly engineered materials such as polymer-infiltrated ceramic (PIC) warrants investigations about its effects on cell metabolism.

An *in vitro* cell viability evaluation may be performed by SEM or enzymatic assay. These assays can measure the metabolic activity of the cellular growth in contact with the materials' surface.[18] The 3-(4,5-dimethylthiazol-2-yl) diphenyltetrazolium bromide (MTT) test is based on the activity of enzymes found in viable cells, such as succinyl dehydrogenase, indicating both the number of viable cells in a sample and the level of metabolic activity.[19,20]

Thus, the aim of this study was to evaluate the *in vitro Streptococcus mutans* adhesion and fibroblast viability on the surfaces of two ceramic materials, zirconia and PIC, indicated for dental restorations and implant components. The null hypothesis was there would be no difference regarding biofilm formation and cell viability between the materials.

METHODS AND MATERIALS

Sample Manufacturing

Presintered blocks of Y-TZP (Vita In Ceram YZ, VITA Zhanfabrick, Bad Säckingen, Germany) and PIC (VITA Enamic, VITA Zahnfabrik) were sectioned into rectangular pieces (4×4×3 mm) with diamond discs (Extec Corp, Enfield, CT, USA) in a precision saw machine (IsoMet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA), under coolant irrigation. The samples (n=32) were polished with SiC paper of decreasing grit size (#400 through #1200) and cleaned in an ultrasonic bath. After polishing with SiC 1200-grit paper, the Y-TZP was sintered in a Zyrcomat furnace (VITA Zahnfabrik). The final dimensions of the blocks (3×3×2 mm) were checked with a digital caliper.

Surface Roughness Analysis

Quantitative analysis of surface roughness was performed in a contact profilometer (Mitutoyo SJ 400, Tokyo, Japan). Three measurements were performed with a distance of 1 mm between them

and a measuring length of 3 mm (n=10). The mean roughness Ra (μm; profile roughness parameter) was then recorded.

Biofilm Adhesion

Ten samples from each material were used to count the CFUs (CFU/mL). Biofilm adhesion was achieved using a modified version of the technique proposed by Anami and others.[1] Standard suspension of *S mutans* (ATCC 35688) containing 10^6 cells/mL was prepared: the bacteria were plated in a brain-heart infusion agar (Difco, Detroit, MI, USA) and incubated for 24 hours at 37°C in a CO_2 chamber. After incubation, the growth was suspended in a sterile physiological solution (0.9% sodium chloride [NaCl]), and the number of suspended cells was counted using a spectrophotometer (B582, Micronal, Sao Paulo, Brazil). The optical density and wavelength parameters used were 0.620 and 398 nm, respectively. These parameters were previously established by means of a standard curve of CFU/mL vs absorbance. Adherence testing was performed in an aseptic environment using a laminar flow chamber. Each specimen was put inside a well of a sterile 24-well polystyrene tissue-culture plate, with 2.0 mL of broth (20 g trypticase, 2 g NaCl, 3 g K_2HPO_4, 2 g KH_2PO_4, 1 g K_2CO_3, 120 mg $MgSO_4$, 15 mg $MnSO_4$, and 50 g $C_6H_8O_7$ dissolved in 1000 mL of distilled water) and 0.1 mL of standardized *S. mutans* suspension. Plates were then sealed and incubated at 37°C for 48 hours in a CO_2 chamber.

Analysis of the Biofilm Formation Using SEM

Two samples from each material were analyzed for biofilm formation. Samples were fixed for one hour in a 2.5% glutaraldehyde solution and dehydrated in ethanol baths (10%, 25%, 50%, 75%, and 90% for 20 minutes and 100% for one hour). Samples were then fixed in a metallic base with carbon adhesive tape (SPI Supplies, West Chester, PA, USA), sputter coated with a gold-palladium alloy (Polaron SC 7620 Sputter Coater, Quorum Technologies, Newhaven, UK; 130 seconds, 10-15 mA, 130 mTorr vacuum, 3.5 nm/min metallization rate, and 80Å Pd-Au layer [approximate]), and observed using SEM (20 kV, Inspect S50, FEI Company, Brno, Czech Republic). We then performed a descriptive analysis of the biofilm formed on the samples.

Analysis of Biofilm Biovolume: CLSM

Five samples from each material were analyzed using CLSM (LSM 510-META, Zeiss, Pleasanton, CA, USA) to assess the biovolume (μ^3/mm^2) of the formed biofilm. Samples were removed from incubation and positioned on glass laminate and stained with the Live/Dead Bac Light Bacterial Viability and Counting Kit (Molecular Probes, Eugene, OR, USA). The kit is composed of two fluorescent staining solutions: SYTO 9 in green color, which stains viable cells (penetrates into cells with intact membranes), and red isopropidium iodide, which stains dead cells (penetrates into cells with injured membranes). The number of optical sections varied according to the biofilm's thickness. COMSTAT software (Technical University of Denmark, Lyngby, Denmark) was used for biovolume analysis.

Cell Viability Evaluation (MTT Test)

Gingival human fibroblasts (FMM-1) were cultured on samples positioned in 24-well polystyrene tissue culture plates. A total of 20,000 fibroblasts were cultured on each sample and maintained in Dulbecco's Modified Eagle Medium (Cultilab, Curitiba, Brazil), supplemented with 10% bovine fetal serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a humid atmosphere with 5% CO_2 for one, three, and seven days. Next, cellular survival was determined by measuring the succinic dehydrogenase activity that indicates mitochondrial function and may be observed by MTT assay (Sigma-Aldrich, St Louis, MO, USA). The activity was quantified by dissolving MTT in 0.1 N NaOH (6.25 v/v%) in dimethyl sulfoxide (Sigma-Aldrich). Optical density readings for the solution were measured in a spectrophotometer (Bio-Tek, Winooski, VT, USA) at 570 nm. The control group was represented by cells without contact with any of the materials. Spectrophotometric data were expressed in percentages of the control group, which was considered as 100%.

Data Analysis

Results for roughness (μm), CFUs, and biovolume were assessed using Student *t*-test ($p<0.05$). Cell viability data were assessed using the Z-test, followed by the Tukey test for mean contrast, in which the materials were compared with the control groups (100%), and analysis of variance (ANOVA) for comparisons between the two materials. Images obtained from SEM and CLSM were qualitatively evaluated.

RESULTS

Roughness, CFU, and Biovolume

The mean values and standard deviations for the roughness, CFU (log_{10}), and biovolume of each material are listed in Table 1. Statistical analysis

Table 1: *Mean Values and Standard Deviations of Roughness, CFU (log_{10}), and Biovolume for PIC and Y-TZP*

	Y-TZP	PIC	*p*-Value*
Roughness (Ra), μm	0.057 ± 0.012	0.132 ± 0.016	<0.0001*
Biofilm adhesion, CFU/mL	8.311 ± 0.237	8.630 ± 0.564	0.1342
Biovolume, $\mu m^3/\mu m^2$	0.0049 ± 0.007	0.0082 ± 0.009	0.4569

** Indicates a statistically significant difference.*

showed that the PIC presented significantly higher Ra values than the Y-TZP.

Qualitative Analysis in SEM and CLSM

The analysis of representative SEM images showed the surface pattern of PIC was rougher than that of Y-TZP (Figure 1), as shown in the Ra values in Table 1. However, both materials showed similar characteristics for *S mutans* adhesion after 48 hours of incubation. The CLSM representative images also demonstrated a similar pattern between the materials with regard to cell viability: viable cells (green) and nonviable cells (red; Figure 2).

An increase in cellular adhesion was observed (Figure 3) depending on the evaluation time, where a larger amount of adhered cells was observed on the PIC surface than on the Y-TZP surface on the first day. However, a similar pattern was observed after 7 days.

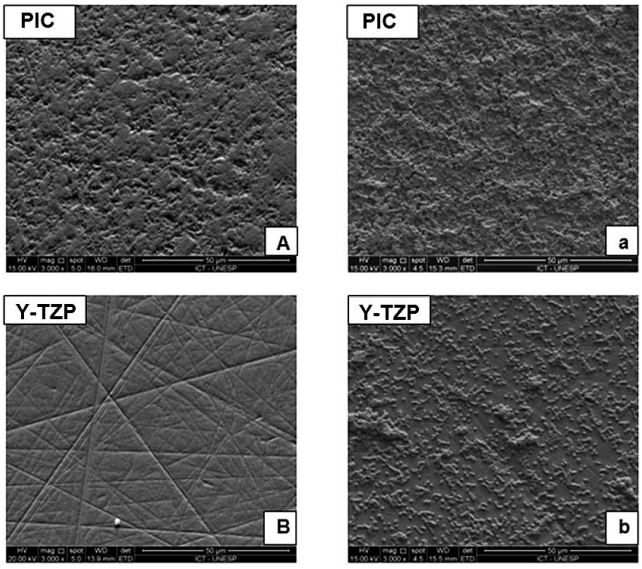

Figure 1. *SEM images of the topography of PIC and Y-TZP (A and B) and the adhesion of* S mutans *on the materials' surfaces (a and b).*

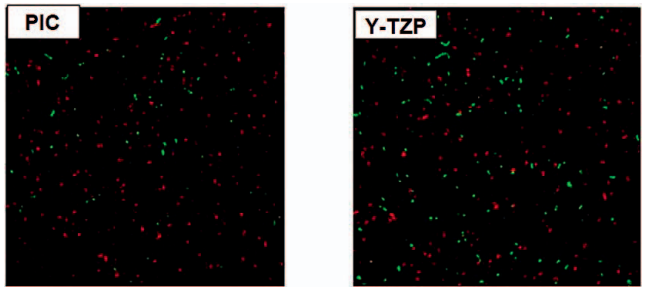

Figure 2. *Confocal laser scanning images used for biovolume and thickness determination of* S mutans *on the surface of the ceramics.*

Cell Viability

The MTT data indicated the materials cannot be considered cytotoxic since the absorbance percentage, which is related to the amount of viable cells after contact with the two materials, was always higher than 50% of the mean found for the control group (Table 2). A comparison of each material to the control group (Z test) indicated that only Y-TZP was statistically different from the control, meaning that the number of viable cells was significantly lower than the number in the control group but yet noncytotoxic. Tukey post hoc test showed that Y-TZP was different from the control group after one day and seven days (Table 3).

When Y-TZP and PIC were compared, ANOVA indicated that these materials were statistically similar (p=0.54). The mean percentages of absorbance after contact with both materials at the evaluation times (one, three, and seven days) are shown in Figure 4.

DISCUSSION

This study evaluated the biological response of two ceramics indicated for indirect dental restorations. The materials presented similar behavior with respect to biofilm adhesion and cellular viability, thus confirming the anticipated hypothesis.

Restorative materials are subjected to biofilm adhesion when placed in the oral environment. The

Table 2: *Mean (%), Standard Deviation, and* p*-Value of Data Obtained for Cellular Viability When Comparing PIC and Y-TZP With the Control Group (100% Absorbance)*

Cytotoxicity	PIC	Y-TZP
Mean	95.06	90.53
Standard deviation	20.30	16.00
p-value	0.39	0.04*

** Indicates a statistically significant difference.*

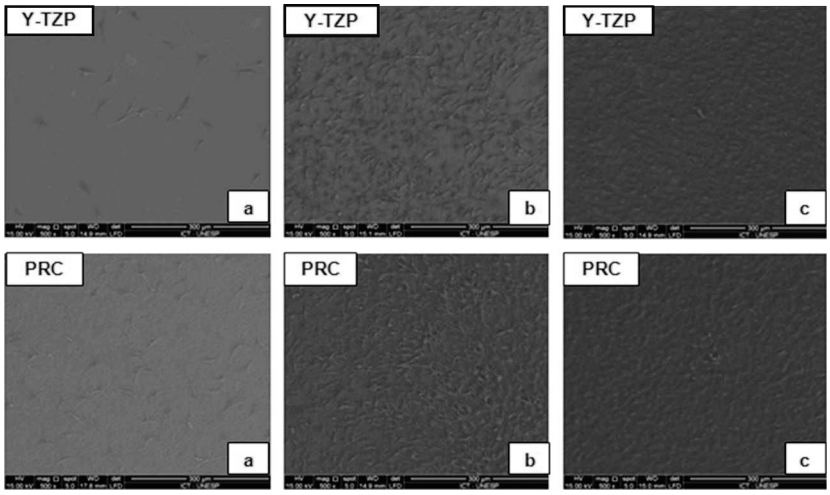

Figure 3. *Representative images of adhered cells on Y-TZP and PIC after one (a), three (b), and seven (c) days. The shape of the cells (narrow and with several extensions) was unchanged until day 3 but could no longer be distinguished on day 7 because of the high number of cells.*

amount of biofilm varies according to the nature of the material[13] and properties such as surface energy and roughness.[21,22] Rough surfaces are more prone to biofilm accumulation than smooth surfaces because the former provide niches where bacteria adhere and grow.[23,24] In general, ceramics are reported to present less bacterial adhesion than other restorative materials.[25] The roughness parameter (Ra) value in this study was significantly higher for the PIC than for the Y-TZP (Table 1). However, the CFU and biovolume values were similar. Thus, according to these quantitative parameters, the roughness did not influence biofilm accumulation, a fact also seen in previous studies.[24,26] On the other hand, rougher ceramic surfaces are more prone to cell adhesion and proliferation.[27,28] Therefore, PIC presented a more favorable surface for cell attachment than zirconia.

Obtaining images via SEM required fixation and dehydration of the biofilm. This procedure can alter the biofilm characteristics but is a well-accepted method for bacteria identification and adherence.[16,21] Using CLSM makes it possible to obtain quantitative data about the formation of biofilm, mean thickness,[29,30] and biovolume (Table 1). This method is considered noninvasive and nondestructive and represents the main tool for evaluation of *in situ* biofilm.[31] The thickness and biovolume parameters, respectively, morphological characteristics of the biofilm and the extracellular material not covalently attached to the cell membrane, were equally expressed in PIC and Y-TZP. Therefore, neither materials affected the structure and capacity of cells to produce the extracellular matrix.

The interpretation of SEM images was difficult, as the topography of PIC was rougher than that of Y-TZP, with the main differences being between microorganism adherence in these materials (Figures 1 and 2). Qualitative CLSM images were important to confirm the SEM findings and showed similarity between the materials in both the amount and the spread of bacteria (Figure 2).

The analysis of the cytotoxic potential of both materials revealed that neither PIC nor Y-TZP were

Table 3: *Mean (%), Standard Deviation, and* p*-Value of Data for Y-TZP of the Cellular Viability Test*

Cytotoxicity	1 Day	3 Days	7 Days
Mean	85.77	109.50	76.31
Standard deviation	8.05	6.82	6.90
p-value	0.03*	0.06	0.006*

** Indicates a statistically significant difference.*

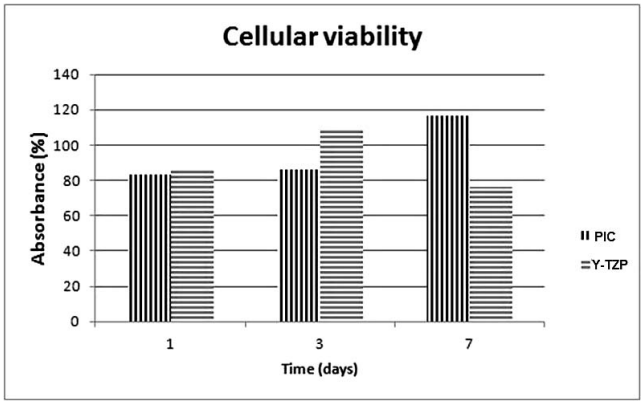

Figure 4. *Representative graph of the mean percentage of absorbance obtained by the MTT test after contact of cells with PIC and Y-TZP at days 1, 3, and 7.*

detrimental to fibroblasts, since both presented cellular viability higher than 50% of the control group (ie, cells not submitted to any material). The number of viable FMM-1 was significantly lower for the Y-TZP group than for the control group (Table 2) at days 1 and 7 (Table 3), but the material was not considered cytotoxic for the cells (90% of cellular viability). Figure 3 indicates that the cellular adhesion was apparently higher on the first day for the PIC material. This was probably a result of time-dependent factors that occur after implantation of the first fibroblasts. One important event is protein adsorption occurring before cell adhesion, as cells adhere and spread quickly on the first days, while the response of upcoming cell layers will be controlled by the protein film.[32] SEM analysis showed homogeneity in cell spreading and intimate contact with the materials after the seven-day analysis, indicating the materials were biocompatible and allowed a high proliferation rate per day.[28]

In long-term clinical studies, zirconia infrastructures were gentle to the periodontium and presented an overall good biological response.[33,34] Our findings suggest that the attachment of fibroblasts to Y-TZP and PIC *in vivo* occurs normally, and the materials themselves should not cause inflammation and bone loss around the peri-implant.[35] These results contradict those of Grenade and others,[36] who differentiated two groups of materials in terms of fibroblasts adhesion, including a Ti-Zi group (more biocompatible) and an eM-PICN group (less biocompatible), with the latter being represented by a PIC. However, the authors call attention to the fact that the hybrid material is not the same commercial brand used herein, and this could partly explain the differences in the results.

Furthermore, PIC contains methacrylate (urethane dimethacrylate and triethylene glycol dimethacrylate) in its composition, which has a significant cytotoxic effect in its uncured form.[37] Although the resin portion of PIC seems highly polymerized, examining the degree of polymer conversion and the effects of monomer elution on cell viability is a must for future studies. Moreover, the biofilm adhesion scenario is more complex *in situ*,[38,39] and additional clinical studies are warranted.

CONCLUSION

Both zirconia and PIC, which are indicated for indirect dental restorations and implant components, were noncytotoxic and presented similar capacity for biofilm adhesion.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institute of Science and Technology at São Jose dos Campos, UNESP.

Conflict of Interest

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

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Diagnostic Value of the Basic Erosive Wear Examination for the Assessment of Dental Erosion on Patients, Dental Photographs, and Dental Casts

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Clinical Relevance

The assessment of the basic erosive wear examination (BEWE) on patients and on dental photographs seems to yield comparable results and therefore may be a suitable tool for longitudinal monitoring of dental erosion. The assessment of BEWE on dental casts may better be used for laboratory techniques.

SUMMARY

Objectives: The aim of this trial was to investigate the diagnostic value of the basic erosive wear examination (BEWE) in clinical use, on dental photographs, and on dental casts over a two-year follow-up period (2013-2015). According to the main hypothesis for longitudinal monitoring of dental erosion, the BEWE is equally reproducible by the three assessment methods.

Methods and Materials: The clinical assessment included intraoral photographic documentation, dental impressions, oral examination, and assessment of BEWE. Clinical assessment of BEWE was done by one blinded examiner, whereas assessment on photographs and dental casts was performed by three calibrated examiners and repeated after 14 days. The three assessment methods were analyzed separately by longitudinal agreement and inter- and intrarater reliability (in-

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traclass correlation coefficient) alongside 95% confidence intervals (CIs).

Results: Comparing the longitudinal data of the years 2013-2015, clinical use and photographs showed no significant difference (p=0.0681-0.9963), whereas the statistical analysis showed a significant difference for dental casts by comparing data from 2013 vs 2014 (p=0.0266) as well as data from 2013 vs 2015 (p=0.0001). Statistical evaluation of overall BEWE showed an intrarater reliability of 0.79-0.91 for photographs and 0.60-0.87 for dental casts. The interrater reliability was 0.77 (95% CI=[0.69; 0.84]) for photographs and 0.63 (95% CI=[0.52; 0.72]) for dental casts.

Conclusion: This investigation showed that in longitudinal clinical monitoring, the assessment of the BEWE on patients and dental photographs yielded comparable results. In addition, based on these findings, the assessment of the BEWE on dental casts showed moderate reproducibility. Therefore, dental casts may be better used for laboratory assessment techniques.

INTRODUCTION

Recent data suggest that oral health, especially caries prevalence, is improving: young adults as well as children experience less caries, and the number of remaining teeth in the elderly population is increasing.[1,2] Therefore, noncarious lesions are brought to focus. In recent years, there has been a rising interest in erosive tooth wear, because the prevalence of dental erosion throughout the European population is high,[3] seems to be increasing,[4] and treatment management is complex because of a multifactorial etiology.

The dentition experiences multifactorial physiological and nonphysiological wear over time, such as attrition, abrasion, or erosion. Dental erosion is a nonbacterial-associated loss of tooth structure and is defined as (partial) demineralization of enamel or dentin by intrinsic[5-7] or extrinsic acids.[8,9] Owing to the multifactorial etiology, the distinguishing acid-induced tissue loss can manifest itself in different ways.[10] Characteristic signs of erosive tooth wear are loss of surface contour, shallow concavities on smooth surfaces, cupping and grooving on occlusal/incisal surfaces, and "proud" restorations.[11] In addition, erosive tooth wear is defined as the accelerated loss of dental hard tissue through the combined effect of erosion and mechanical wear (abrasion and attrition).[4] Thus, early diagnosis is particularly challenging, because of the multifactorial combination of wear.

As pathological tooth wear is defined as an unacceptable level of progressive wear,[12] there is both a clinical and scientific need to be able to measure and monitor erosive tooth wear, and the literature abounds with many methods. Today, practitioners are given myriad different diagnostic tools and grading scales for qualitative and quantitative assessment of dental erosion to be used either chairside, on dental casts, on three-dimensional (3D) models, and/or on intraoral photographs. The four most commonly used evaluation systems are the tooth wear index,[13] the Eccles index,[8] the Lussi index,[14] and the basic erosive wear examination (BEWE).[10,15] They vary, for example, in type of assessment, diagnostic criteria, thresholds, and choice of teeth; therefore, a wide range of prevalence of erosive wear (4%-82% in adults) is present in the literature. Finally, this abundance of diagnostic tools and grading scales results in noncomparability of the different indices.[4,16] In 2008, Bartlett and others[15] introduced the BEWE. It was developed to provide a simpler way to monitor and record the severity and progression of erosive tooth wear in general practice as well as in education and research and in turn increased the awareness of dental erosion. Furthermore, the BEWE aims to be reproducible and comparable to other more discriminative indices. It is supposed to be used chairside, on dental casts and dental photographs in equal measure.[15]

Since its introduction, the scientific community has approved the BEWE for clinical use and epidemiological research, although the data concerning reliability and validity are still rare. Moderate to good reliability has been reported for chairside examination.[17-19] As far as we know, only one study has reported the reliability of scoring dental erosion on intraoral photographs. Mulic and others[19] showed the BEWE to be acceptable for evaluating erosive wear on photographs, being possibly on par with the clinical examination. Originally, it was developed as a tool for grading dental casts as well[15]; however, at the moment, there is only one study demonstrating its moderate reliability on dental casts.[17] A Finnish study published data on the measure's reliability on digital 3D models, finding the BEWE index to be reliable but that it may not be entirely comparable with the clinical assessment of erosive wear.[20]

To the best of our knowledge, no longitudinal study has been conducted comparing the diagnostic value of the BEWE for assessing and monitoring

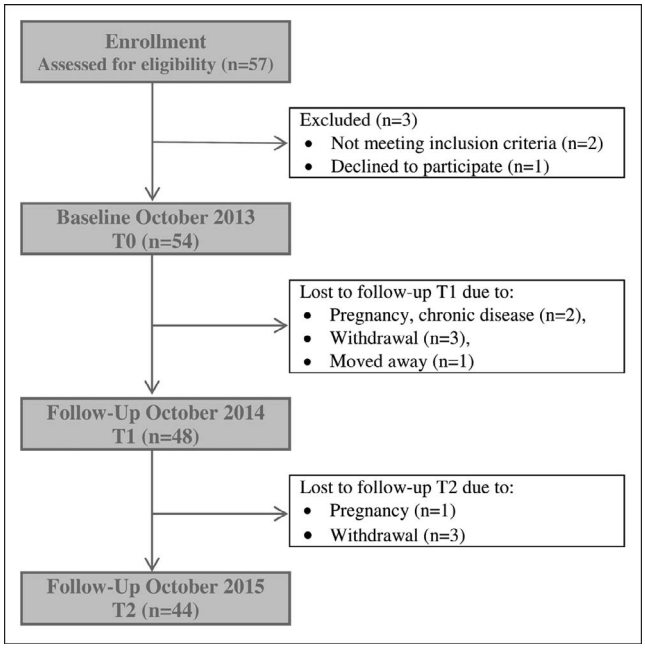

Figure 1. *Flowchart dropouts.*

dental erosion on patients, on photographs, and on dental casts.

In consideration of the scarce literature on the reliability of the BEWE index, the aim of this study was to assess the diagnostic value of the BEWE in clinical use, on dental casts, and on dental photographs over a two-year follow-up period. Accordingly, the null hypotheses were that 1) there is no longitudinal agreement of the three diagnostic methods and 2) that in a comparison of the three diagnostic methods, the BEWE is not reproducible by different examiners in longitudinal follow-up.

METHODS AND MATERIALS

Clinical Investigation

This investigation is part of a monocentric, prospective clinical cohort trial that was conducted after obtaining approval from the local medical ethics committee (S-566/2012). The study was registered at the German Clinical Trials Registry Platform (DRKS00005019) that is linked to the International Clinical Trials Registry Platform of the World Health Organization. In this clinical investigation, a formerly identified group of patients with a moderate to high risk of dental erosion (endurance athletes, 18 years of age or older, in good general health, not pregnant/nursing, not restricted in practicing oral hygiene, no signs of severe oral diseases, no dental staff or dental student, no intake of antibiotics during the past 30 days, no participation in another clinical trial within 30 days) was chosen.[21] Written, informed consent was obtained from all participants. While in 2013 there were 54 participants in the investigation, the number was reduced to 48 in 2014 and even further to 44 in 2015 because of subjects abandoning the study (see Figure 1).

The workflow is shown in Table 1. Clinical examination was performed between October 2013 and October 2015. Every year, one blinded examiner (TW) performed the standardized clinical examination. The study protocol included medical history, intraoral examination, assessment of dental erosion (BEWE), standardized photographs, and dental impressions (Impregum Penta Soft, 3M ESPE, Neuss, Germany). After professional tooth cleaning, the intraoral examination was performed by blow drying the teeth and using a dental operating light, an additional portable light-emitting diode illumination system (Bajohr LED Powerlight, Bajohr GmbH & Co. KG, Einbeck, Germany), binocular loupes (magnification 2.5×), plain mirrors, and diagnostic probes.

Clinical Assessment and Calibration

The clinical assessment and calibration are shown in Table 1. Prior to the study, all three examiners (CF, TW, and SF) respectively completed an e-learning training program successfully educating them in how to use the BEWE and ensuring they had similar theoretical knowledge (http://elearningerosion.com/de/elearning_erosion.html). To standardize the clinical investigation, 16 patients were assessed by two examiners (CF and TW) using the BEWE index (see Table 1). In the exact same manner, the BEWE was assessed on each of 20 photographs and dental casts by all three examiners (CF, TW, and SF; see Table 1).

BEWE

For assessment of erosive tooth wear, the BEWE was performed. The severity of erosive tooth wear was graded by a four-level scoring system (0-3) relying on appearance (see Table 2). In this investigation, the sextant-scoring method was performed. This means that the examination was conducted on all teeth, but only the surface with the highest score within one sextant was recorded. Fully restored surfaces were excluded. In case of doubt, the examiners were told to choose the lower of two scores. Once all the sextants had been assessed, an overall score was calculated for each patient by adding the highest

Table 1: *Flowchart Calibration and Assessment*

Clinical calibration n = 16	Calibration for standardized intraoral photographs n = 20	Calibration for dental casts n = 20
October 2013: 1. clinical investigation + dental impression + photographic documentation n = 54	1. assessment of photographs 2013 (n = 54) 1. assessment of dental casts 2013 (n = 53)	
	2. assessment of photographs 2013 (n = 54) 2. assessment of dental casts 2013 (n = 53)	
October 2014: 2. clinical investigation + dental impression + photographic documentation n = 48	1. assessment of photographs 2014 (n = 48) 1. assessment of dental casts 2014 (n = 47)	
	2. assessment of photographs 2014 (n = 48) 2. assessment of dental casts 2014 (n = 47)	
October 2015: 3. clinical investigation + dental impression + photographic documentation n = 44	1. assessment of photographs 2015 (n = 44) 1. assessment of dental casts 2015 (n = 44)	
	2. assessment of photographs 2015 (n = 44) 2. assessment of dental casts 2015 (n = 44)	

scores of each sextant and matching the result to an individual risk level.[15]

Assessment on Photographs and Dental Casts

The workflow is shown in Table 1. To guarantee standardization of intraoral photographs, the shutter speed of the camera was set to 1/125s and the aperture value to f27. The teeth were blow dried, and dental photo mirrors helped to ensure a comprehensive view of either the mandible or maxilla. For the assessment of the BEWE, at least one overview of the frontal and lateral aspect of a jaw were taken.

In 2013, 53 dental casts were made and 54 intraoral images taken; in 2014, 47 dental casts and 48 intraoral images were taken; and in 2015, 44 dental casts and intraoral images alike were taken (Table 1).

Three blinded examiners (CF, TW, and SF) assessed erosive tooth wear by using the BEWE index on dental casts and standardized intraoral photographs. By reassessing the dental casts and standardized photographs again after 14 days, the intrarater reliability was evaluated (Table 1).

Table 2: *Visible Criteria of Basic Erosive Wear Examination (BEWE) Coding System*

Code	Visible Criteria
0	No sign of erosive tooth wear/surface loss
1	Initial loss of surface texture
2[a]	Distinct defect, hard tissue loss <50% of the surface area
3[a]	Hard tissue loss ≥50% of the surface area

[a] *In scores 2 and 3, dentin is often involved.*

Statistical Analysis

The data were analyzed using descriptive statistics evaluating means and standard deviation. The primary measurement outcome was the reliability of the assessment of erosive tooth wear on dental casts and standardized intraoral photographs. Thus, the statistical analysis of the BEWE sextant score was evaluated by calculating the cumulative BEWE sum score using the results of the assessment of dental erosion on dental casts and photographs. The clinical examination was used as validation and considered as the gold standard. The extent of inter- and intrarater agreement as well as the intermethod agreement was analyzed using the intraclass correlation coefficient (ICC). The ICC values were interpreted as suggested: <0.5, poor; 0.5-0.75, moderate; 0.75-0.90, good; and 0.90-1, excellent.[22]

Table 3: *Assessment Results of the BEWE on the Three Diagnostic Methods From 2013 to 2015 Are Depicted in Mean Values (MV) and Standard Deviations (SD). Statistically significant differences were calculated between the results of the clinical assessment and, respectively, the evaluations of photographs and dental casts (*p*-value based on* t*-test for dependent samples).*

	Year	n	MV	SD	*p*-value
2013					
	BEWE score patients	54	8.396	± 3.047	—
	BEWE score photographs	54	9.25	± 2.855	0.0681
	BEWE score dental casts	54	8.698	± 3.058	0.5209
2014					
	BEWE score patients	48	7.958	± 3.696	—
	BEWE score photographs	48	8.802	± 2.692	0.1424
	BEWE score dental casts[a]	48	9.378	± 3.038	**0.0167[a]**
2015					
	BEWE score patients	44	9.295	± 3.137	—
	BEWE score photographs	44	9.293	± 2.471	0.9963
	BEWE score dental casts	44	9.869	± 2.843	0.2733

[a] *Comparing the cumulative BEWE score of dental casts in 2014 with the corresponding BEWE score of the clinical assessment, there was a statistically significant difference.*

BEWE scores were also compared between assessment methods and time points using (descriptive) *t*-tests for dependent samples. *p*-values smaller than 0.05 were regarded as statistically significant. The analysis was carried out using R 3.2.2 (http://r-project.org) and the package "psych."

RESULTS

General Data

Recruitment of patients was done between March and October 2013. The baseline examination took place in October 2013. Follow-up appointments were carried out in October 2014 and 2015. The workflow is shown in Table 1.

Of 54 participants, 13 were female and 41 were male. At the beginning of the trial, the mean age of the participants was 36.53 ± 9.49 years (range, 20-60 years).

The annual dropout rate was 11.11% from 2013 to 2014 and 8.33% from 2014 to 2015, as shown in Figure 1. Some of the patients were either university students who moved away or were unwilling to participate in the study any longer. Two participants had to discontinue their participation because of pregnancy and one due to chronic disease (Figure 1).

Table 4: *Statistically Significant Differences Were Calculated From the Results of Applying the BEWE in Clinical Examinations on Photographs and Dental Casts, Respectively (*p*-Value Based on* t*-Test for Dependent Samples)*

	Clinical Examination, *p*-Value	Photographs, *p*-Value	Dental Casts, *p*-Value
2013 vs 2014	0.52	0.1038	**0.0266***
2013 vs 2015	0.1579	0.8732	**0.0001***
2014 vs 2015	0.064	0.0697	0.1116

** Statistically significant difference.*

Calibration

The interrater reliability regarding clinical examination was good (0.88; 95% confidence interval: 0.70-0.95). The agreement between all the raters regarding intraoral photographs was moderate (0.60; 95% confidence interval: 0.23-0.81), and the interrater agreement for dental casts was good to excellent (0.90; 95% confidence interval: 0.77-0.96).

Longitudinal Agreement of the Three Methods

The results of the cumulative BEWE for all three methods are depicted as a mean value and standard deviation in Table 3. At baseline, the mean cumulative BEWE of the clinical examination was 8.396 ± 3.047 and reached 9.295 ± 3.137 after two years. The mean cumulative BEWE of intraoral photographs and dental casts at baseline were 9.25 ± 2.855 and 8.698 ± 3.058; after two years they were 9.293 ± 2.471 and 9.869 ± 2.843. Comparing the cumulative BEWE of photographs and dental casts with the corresponding BEWE of clinical examination, the assessment of dental erosion on dental casts showed a statistically significant difference in the 2014 follow-up examination (p=0.0167; Table 3).

The results of the longitudinal comparison of the three diagnostic methods are depicted in Table 4 and Figure 2. Between 2013 and 2015, the assessment of the BEWE during clinical examination showed no statistically significant increase in dental erosion (p=0.1579; see Table 4 and Figure 2). In addition, no significant increase in dental erosion could be observed in the mean BEWE score of intraoral photographs over a period of two years (p=0.8732). On the contrary, the mean BEWE score for the assessment on dental casts showed a linear trend and increased significantly after one year (p=0.0266); in addition, a highly significant increase in dental erosion was detected after two years (p=0.0001; see Figure 2 and Table 3). Null hypothesis 1 could in parts be rejected.

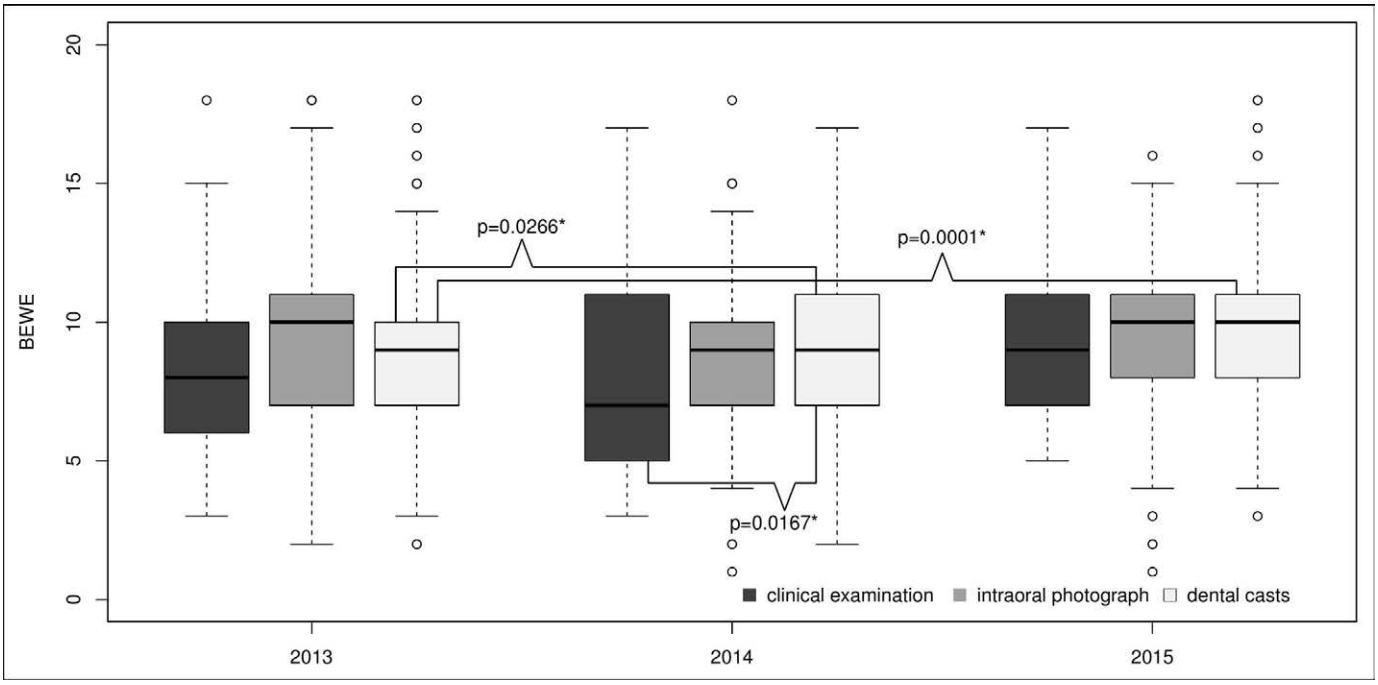

Figure 2. *Box plot of cumulative BEWE score sum of clinical examination, intraoral photographs, and dental casts over time (2013-2015). *Significant difference in the BEWE was shown for the dental casts of 2013 vs 2014 (*p*=0.0266 for paired* t*-test) and 2013 vs 2015 (*p*=0.0001 for paired* t*-test).*

Intrarater Reliability

The results of overall intrarater reliability are depicted in Table 5 and visualized in Figure 3. BEWE was assessed on intraoral photographs and dental casts, respectively. After two weeks, the assessment of the same photographs and dental casts was repeated. In the year 2015, the intrarater reliability on intraoral photographs was good to excellent (0.79-0.91), whereas the intrarater reliability performing BEWE on dental casts varied from moderate to good (0.60, 0.67, and 0.87). In the maxilla, the results varied from good to excellent (0.78-0.91) on intraoral photographs and from moderate to good (0.51-0.78) on dental casts. In the

Table 5: *Intrarater Reliability Was Calculated for the Assessment of the BEWE (Complete Dentition, Maxilla, and Mandible) on Photographs and Dental Casts of the Years 2013 to 2015. The mean cumulative BEWE sum score of intraoral photographs and of dental casts were compared within one rater.*

	Intrarater Reliability on Intraoral Photographs	95% CI	Intrarater Reliability on Dental Casts	95% CI
Complete dentition				
Rater 1	91.51%	[84.93%; 95.3%]	**67.79%**[a]	[47.85%; 81.12%]
Rater 2	85.97%	[75.66%; 92.13%]	87.25%	[77.77%; 92.87%]
Rater 3	79.35%	[65.08%; 88.23%]	**60.95%**[a]	[38.33%; 76.71%]
Maxilla				
Rater 1	91.56%	[85.02%; 95.33%]	**66.77%**[a]	[46.68%; 80.34%]
Rater 2	85.34%	[74.62%; 91.76%]	78.90%	[64.59%; 87.88%]
Rater 3	78.60%	[63.92%; 87.78%]	**51.21%**[a]	[25.90%; 70.00%]
Mandible				
Rater 1	**73.10%**[b]	[55.57%; 84.43%]	**62.29%**[b]	[40.47%; 77.45%]
Rater 2	**70.71%**[b]	[52.06%; 82.95%]	81.98%	[69.4%; 89.72%]
Rater 3	**72.63%**[b]	[54.88%; 84.15%]	**72.28%**[b]	[54.61%; 83.82%]

[a] *After 2 weeks, the assessment of dental erosion on dental casts merely showed moderate reliability.*
[b] *After 2 weeks, the assessment of dental erosion on dental casts and intraoral photographs showed moderate reliability.*

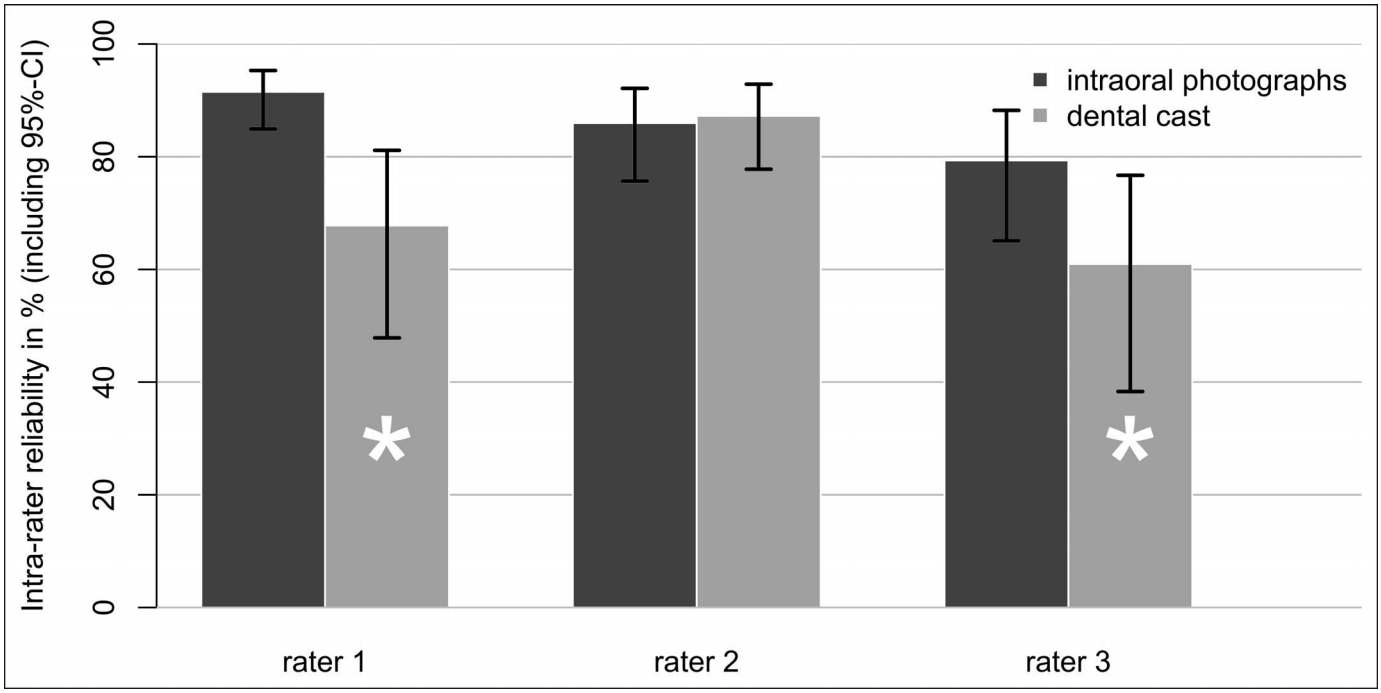

Figure 3. *Comparison of the intrarater reliability of rater 1 (CF), rater 2 (TW), and rater 3 (SF). *After two weeks, the assessment of dental erosion on dental casts merely showed moderate reliability.*

mandible, results showed a moderate intrarater reliability on intraoral photographs (0.70-0.73), and a moderate to good intrarater reliability on dental casts (0.62-0.81; see Table 5).

Interrater Reliability

The overall results of all three raters for the assessment of the BEWE on photographs and dental casts were compared with each other and are depicted in Table 6. Interrater agreement could not be calculated for the assessment of the BEWE on patients, because the clinical study protocol allowed only one blinded examiner. The statistical evaluation showed an interrater reliability on intraoral photographs between 0.67 and 0.84 and therefore was rated good. On the other hand, the interrater reliability on dental casts was moderate (0.57-0.67). In the maxilla, the statistical analysis showed a moderate to good interrater reliability on intraoral photographs (0.71-0.83). The interrater reliability on dental casts was moderate (0.55-0.66). In the mandible, a moderate to good interrater reliability on intraoral photographs (0.56-0.75) and a poor to moderate interrater reliability on dental casts (0.45-0.60) was shown.

DISCUSSION

Erosive tooth wear is a clinical condition that calls for the increased attention of the dental community and provides a challenge in forming cooperation with other medical specialties.[23] The management of erosive tooth wear needs to be preceded by an accurate diagnosis and documentation of its severity. Accurate techniques for measuring erosive tooth

Table 6: *Overall Interrater Reliability Was Calculated for the Assessment of the BEWE on Photographs and Dental Casts of the Years 2013-2015. The mean cumulative BEWE sum score of intraoral photographs and dental casts were compared between the three raters (t-test).*

	Intraoral Photographs	95% CI	Dental Casts	95% CI
Rater 1 vs rater 2	67.74%	[54.48%; 77.7%]	57.97%	[42.09%; 70.42%]
Rater 1 vs rater 3	77.58%	[67.58%; 84.78%]	67.67%	[54.39%; 77.65%]
Rater 2 vs rater 3	84.72%	[77.5%; 89.76%]	64.70%	[50.57%; 75.47%]
Rater 1 vs rater 2 vs rater 3	**77.18%**[a]	[69.37%; 83.63%]	**62.87%**[b]	[52.00%; 72.48%]

[a] *The evaluation of intraoral photographs showed good interrater reliability.*
[b] *The evaluation of the dental casts showed only moderate interrater reliability.*

surface loss have yet to be successfully adapted to clinical settings.[17] The European Federation of Conservative Dentistry recommends the BEWE index for classifying erosive tooth wear.[23] In 2008, Bartlett and others[15] presented the BEWE with the intention to initiate the development of an international accepted index, but little research has been done so far.

This study is the first investigation to evaluate the reliability of the BEWE index on dental casts, dental photographs, and in clinical examination, respectively, and therefore aims to contribute to the continued evaluation and further development of the BEWE system as an international standard. The study compares erosive wear assessment and monitoring using outcomes of analyses of dental casts, photographs, and clinical examination over a two-year period. Therefore, this investigation additionally assessed the longitudinal agreement of three diagnostic methods over a limited period of time. To the best of our knowledge, no comparable study exists in the literature so far.

Previously, the literature has shown the BEWE index to be valid and acceptable for recording dental erosion clinically,[17-19] on dental casts,[17] on 3D models,[20] as well as on dental photographs.[19] Nevertheless, publications engaging the BEWE index are few and far between. The particular strength of our investigation lies in the same three examiners assessing erosive tooth wear on photographs as well as on dental casts in the years 2013-2015. Because of the study protocol, the clinical examination could be performed by only one of the three calibrated examiners. To standardize the assessment, all three examiners were subjected to calibration. This seems to be important and may be an attempt to avoid diagnostic uncertainties.[19,24] In addition, the moderate to excellent interrater reliability during the calibration process (0.60-0.90) supports this fact.

With special regard to the different diagnostic methods, Hove and others[24] reported low to moderate intermethod agreement when assessing dental erosion (κ_w=0.43-0.52 using the VEDE index) by indirect methods, such as on dental casts and photographs, in comparison with clinical examination. On the other hand, where the BEWE index is concerned, it was recently shown that it can be used reliably both at the chair side and on photographs, and furthermore, when comparing the results of the latter two applications, equal grades were obtained.[19] The data of the present study support this assumption and showed no statistically significant difference between scoring dental erosion on patients and on photographs using the cumulative BEWE sum score (0.0681/0.1424/0.9963; Table 3; Figure 2). The BEWE sum score was evaluated over time using the results of the baseline examinations in 2013 and follow-up appointments (2014 and 2015), respectively (Table 3). Comparing the cumulative BEWE sum score of dental casts in 2014 with the corresponding BEWE sum score of the clinical assessments, a statistically significant difference was found (p=0.0167; Table 3). In addition, the analysis of the longitudinal agreement of the three methods showed that no statistically significant difference was detected between clinical examination and the assessment of dental photographs, although dental photographs showed a decrease in severity between 2013 and 2014. However, results of assessing erosive tooth wear on dental casts showed highly significant differences over the whole observation period (p=0.001) as well as between baseline examination and first follow-up appointment (p=0.0266; Figure 2; Table 4). According to this, the longitudinal assessment of the BEWE is considered to be more reliable on intraoral photographs than on dental casts. Therefore, photographs have been shown to be superior when using BEWE as an assessment tool during longitudinal follow-up. Dental casts should be used for longitudinal documentation and various methods of laboratory analysis of dental erosion.

In the present study, the level of intrarater reliability showed a good to excellent intrarater agreement in the assessment of dental erosion on intraoral photographs using the BEWE index. The raters graded the lesions with very high consistency (0.79, 0.85, 0.91), and thus it appears that the use of the BEWE index on intraoral photographs seems an eligible tool, when it comes to the assessment of erosive tooth wear and its progression. The two-week gap was considered to be an adequate period of time to avoid bias by the first assessment. However, little literature can be found considering intraexaminer agreement on photographs. The present findings coincide with current studies, which found that the assessment of the BEWE index on photographs (κ_w=0.63; κ_w=0.66-0.95) was good to excellent.[19,24] On the other hand, when assessing erosive tooth wear by using the BEWE index on dental casts, the intrarater reliability tends to show a moderate to good agreement (0.60, 0.67, 0.87; see Table 5 and Figure 3). These findings are in line with the present literature as well, which assumes that assessing erosive tooth wear on dental casts by means of the BEWE might be more difficult: moderate intrarater reliability (κ_w=0.57 and κ_w=0.60) was shown.[17,24]

With special regard to the interrater reliability, recent findings could also be confirmed. By comparing the results of the raters derived from applying the BEWE on dental casts as well as on photographs, moderate to good interrater reliability was shown for intraoral photographs (0.67-0.84), whereas the assessments of the raters concerning the use of the BEWE on dental casts yielded moderate results (0.57-0.67; see Table 6). However, there is evidence that erosive tooth wear can be graded independently of rater using the BEWE index on intraoral photographs and with a higher consistency than on dental casts. Mulic and others[19] detected similar reliability on photographs (κ_w=0.58-0.91). The wider range of results by Mulic could be a result of the lack of proper validation.[19]

In contrast to the suggestion of Bartlett and others,[15] this study claims that in longitudinal observation, the performance of BEWE on dental casts is inferior than on intraoral photographs. One of the advantages of intraoral photographs might be the display of shade and color. On one hand, restorations are detectable more easily, and on the other hand, the appearance of smooth, dull, and/or shiny surfaces can be detected on photographs more easily. As a matter of fact, changes in morphology do occur only in the more advanced stages.[25] The BEWE system does not distinguish between enamel loss and exposed dentin, which could be regarded as a way to avoid diagnostic uncertainties[15] and furthermore allows it to be used for the assessment of dental erosion on dental casts. Dental casts may have the ability to give good quantitative measurements[24] and offer the opportunity to inspect the teeth three-dimensionally from many angles. However, as it is reflected on the minor intermethod agreement, the type of surface loss (eg, caries, abfraction, attrition, abrasion, erosion) on dental casts might be difficult to differentiate correctly because of the lack of color and shade.

CONCLUSION

Based on these findings, it seems that there is evidence that the diagnostic value of the BEWE in longitudinal assessment and monitoring of erosive tooth wear yields comparable results for intraoral photographs and the clinical examination. The assessment of the BEWE on dental casts was not entirely comparable in this setting. In addition, the BEWE has been shown to be independent of rater using BEWE on photographs and on patients. Because of the moderate reproducibility, dental casts are inferior to clinical examination and intraoral photographs. Therefore, dental casts should be used for longitudinal clinical documentation, profilometry, and other 3D measurement techniques of erosive tooth loss.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Heidelberg, Medical Faculty, Ethics Committee (German: Ethikkommission der Medizinischen Fakultät Heidelberg, Universitaet Heidelberg, Germany). The approval code for this study is S-566/2012.

Author Contributions

TW wrote the first draft of the publication, is responsible for the content as guarantor, and conducted the clinical examination. CF planned the study and provided expertise in the interpretation of the data and final manuscript. CF, SF, and TW conducted the assessment on dental casts and standardized intraoral photographs. DS carried out the data analysis. DW co-developed the study and worked on subsequent publication drafts.

Conflict of Interest

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

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Effect of Immediate Dentin Sealing and Surface Conditioning on the Microtensile Bond Strength of Resin-based Composite to Dentin

CRG van den Breemer • M Özcan • MS Cune
AP Almeida Ayres • B Van Meerbeek • MMM Gresnigt

Clinical Relevance

For partial indirect restorations, immediate dentin sealing is recommended, as bond strength remains stable over time.

SUMMARY

This study evaluated the microtensile bond strength (μTBS) of resin-based composite (RBC) to dentin after different immediate dentin sealing (IDS) strategies and surface-conditioning (SC) methods and on two water storage times. Human molars (n=48) were randomly divided into eight experimental groups involving four different IDS strategies—IDS-1L with one layer of adhesive, IDS-2L with two layers of adhesive, IDS-F with one layer of adhesive and one layer of flowable RBC, and DDS (delayed dentin sealing) with no layer of adhesive (control)—and two different SC methods—SC-P with pumice rubbing and SC-PC with pumice rubbing followed by tribochemical silica coating. The μTBS test was

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performed after one week and after six months of water storage, being recorded as the "immediate" and "aged" μTBS, respectively. Composite-adhesive-dentin microspecimens (0.9×0.9×8–9 mm) were stressed in tension until failure to determine the μTBS. Failure mode and location of failure were categorized. Two-way analysis of variance was applied to analyze the data for statistically significant differences between the experimental groups ($p<0.05$). Two-way analysis of variance revealed no significant differences between the one-week μTBS specimens for IDS strategy ($p=0.087$) and SC methods ($p=0.806$). However, the interaction of IDS strategy and SC methods appeared statistically significant ($p=0.016$). The six-month specimen evaluation showed no significant difference in μTBS for SC ($p=0.297$) and SC/IDS interaction ($p=0.055$), but the μTBS of the IDS strategies differed significantly among them ($p=0.003$). For tribochemical silica-coated IDS, no significant effect of aging on μTBS was recorded ($p=0.465$), but there was a highly significant difference in μTBS depending on the IDS strategy ($p<0.001$). In addition, the interaction of IDS and aging was borderline statistically significant ($p=0.045$). The specimens failed mainly at the adhesive-dentin interface for all experimental groups. Dentin exposure during clinical procedures for indirect restorations benefits from the application of IDS, which was shown to result in higher bond strength. No significant differences were found between cleaning with solely pumice or pumice followed by tribochemical silica coating.

INTRODUCTION

In restorative dentistry, one of the primary goals is to preserve tooth tissue. Removing large amounts of dental structure adversely affects the pulp and may lead to pulp damage.[6,20] When it comes to restoring posterior teeth with large cavities, partial indirect restorations in glass-ceramic or feldspathic porcelain may be indicated. The literature reveals that such restorations have a survival rate of 91% in 10 years.[24] The cause of failure involves fractures (4%), endodontic complications (3%), secondary caries (1%), and debonding (1%).[24] Fractures and debonding are often seen in cases where restorations are bonded to dentin.[13] Creating a strong bond to dentin that is durable over time is more challenging than creating a bond to enamel because of dentin's intrinsic hydrophilic nature.[3] An inadequate seal of dentin by the adhesive may cause postoperative sensitivity, marginal staining, and recurrent caries. Hence, the survival and success of (partial) indirect restorations is often related to the remaining quantity and quality of enamel.[15]

In order to improve bond strength to dentin, Pashley and others introduced the so-called dual bonding technique, which consists of the application of two layers of adhesive resin onto dentin.[26] Applying an adhesive layer directly after crown preparation protects the pulp from bacterial invasion, reduces postoperative sensitivity, and increases bond strength. Other studies revealed that multiple adhesive layers can further improve bond quality and strength.[8,17,19,27] The purpose of sealing dentin directly after preparation is to avoid surface contamination during the temporary phase and to protect dentin by hybridization, thus avoiding sensitivity and preventing water uptake. This requires that the adhesive be light cured immediately, which is commonly not recommended at the time of cementation to avoid restoration fit problems.[32]

In 2005, this concept evolved to immediate dentin sealing (IDS). Prior to luting in the second visit, one commonly recommends decontaminating the IDS by tribochemical silica coating.[21,22] This not only microroughens the surface, thereby improving micromechanical interlocking, but also cleans the surface and enables chemical copolymerization of the resin-based cement with the IDS.[1,33,37] Falkensammer and others[14] concluded that polishing and airborne particle abrasion with silica-coated alumina (Al_2O_3) and glycine are equally efficient methods of conditioning IDS surfaces. Other studies showed that soft air abrasion,[34] airborne particle abrasion with Al_2O_3,[11,22,23] or fluoride-free pumice paste systems[5,12,21] resulted in the highest bond strength. However, it is unknown which method is most suitable for conditioning IDS prior to cementation.

Results from a recent systematic review indicated that the effect of IDS on bond strength is tested mainly by using a microtensile bond strength (μTBS) approach.[38] The μTBS test is generally accepted as one of the most valid bond-strength tests because it is performed perpendicular to the adhesive interface.[10,35] Using a μTBS test, a more favorable stress distribution is achieved, resulting from the small specimen size.

The objective of this study was to compare different IDS applications and surface conditioning

Table 1: *Brand, Manufacturer, Main Chemical Composition, and Batch Number of the Different Products Used (in Alphabetical Order)*

Product (Manufacturer)	Composition	Batch Number
CoJet sand (3M, Seefeld, Germany)	Aluminum oxide (Al_2O_3) particles coated with silica (particle size: 30 μm)	446317/534151
Durelon (3M)	Powder: zinc oxide, stannous fluoride, tin dioxide	514362
	Liquid: water, polyacrylic acid	510198
Enamel plus HFO UD2 (Micerium, Avegno, Italy)	1,4-Butandioldimethacrylate, urethane dimethacrylate, Bis-GMA	2015007203
ESPE-Sil (3M)	Ethyl alcohol, 3-methacryloxypropyl- trimethoxysilane, methyl ethyl ketone	598868
Glycerin Gel K-Y, (Johnson & Johnson, Sezanne, France)	Purified water, glycerin, methylparaben, propylparaben, propylene glycol, hydroxyethylcellulose, dissodium phosphate, sodium phosphate, tetrasodium EDTA	2744V
Grand IO Flow (VOCO, Cuxhaven, Germany)	1,6-Hexanediylbismethacrylate, BIS GMA, triethylene glycol dimethacrylate	1512472
Monobond Plus (Ivoclar Vivadent, Schaan, Liechtenstein)	Ethanol, 3-trimethoxysilsylpropyl-methacrylate, methacrylated phosphoric acid ester	T07775
Optibond FL (Kerr, Orange, CA, USA)	Primer: HEMA, GPDM, PAMM, ethanol, water, photoinitiator	5534310
	Adhesive: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, filler, photoinitiator	5594053
Total Etch (Ivoclar Vivadent)	37% phosphoric acid (H_3PO_4)	L049

Abbreviations: Bis-GMA, bisphenol A-glycidyl methacrylate; EDTA, Ethylenediaminetetraacetic acid; HEMA, Hydroxyethylmethacrylate; GPDM, glycerophosphoric acid dimethacrylate; PAMM, phthalic acid monomethacrylate;TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate

(SC) methods by determining the bond strength to dentin with and without water storage aging. The null hypotheses tested were that (1) there is no significant difference in μTBS among the four IDS strategies investigated, (2) among the two SC methods, and (3) for the "immediate" (one-week) and "aged" (six-month) μTBS.

METHODS AND MATERIALS

Specimen Preparation

Human molars (n=48) were collected, stored in distilled water, and used at maximum one month postextraction. Each specimen was embedded in gypsum to facilitate handling. The occlusal coronal third of the crown was removed with a diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA), thereby exposing a flat midcoronal dentin surface. The dentin surfaces were verified for the absence of enamel and/or pulp tissue exposure using a stereomicroscope (Wild M5A, Wild, Heerbrugg, Switzerland).

Study Design

The flattened specimens were randomly divided into a total of eight experimental groups involving four different IDS strategies—IDS-1L with one layer of adhesive, IDS-2L with two layers of adhesive, IDS-F with one layer of adhesive and one layer of flowable resin-based composite (RBC), and DDS (delayed dentin sealing) with no layer of adhesive (control)—and two different SC methods—SC-P with pumice rubbing and SC-PC with pumice rubbing followed by tribochemical silica coating. The μTBS test was performed after one week and after six months of water storage, being recorded as the "immediate" and "aged" μTBS, respectively.

IDS

The brand, manufacturer, main chemical composition, and batch number are detailed in Table 1 for the different products used in this study.

Regarding IDS-1L, the flat dentin was etched for 15 seconds with 37% phosphoric acid (Total Etch, Ivoclar Vivadent, Schaan, Liechtenstein) and rinsed thoroughly with a water-air spray for 15 seconds. The surface was air-dried but not desiccated for 3 seconds, after which a primer (Optibond FL Primer, Kerr, Orange, CA, USA) was applied with a light brushing motion for 15 seconds and gently air-dried for 10 seconds and suction dried for 15 seconds. A thin layer of heated (40°C) adhesive resin (Optibond FL Adhesive, Kerr) was applied onto the surface using a light brushing motion for 15 seconds and

photopolymerized for 10 seconds (Bluephase Style, Ivoclar Vivadent) (>1000 mW/cm^2). Regarding IDS-2L, the same procedures were performed with the addition that a second layer of adhesive was likewise applied as described above for the first adhesive layer. Regarding IDS-F, the same procedure was performed as for IDS-1L with the addition that a flowable RBC (Grand IO Flow, VOCO, Cuxhaven, Germany) was additionally applied in a thin layer of about an average thickness of 1 mm and separately light cured for 40 seconds. Finally, in all groups, glycerin gel (K-Y, Johnson & Johnson, Sezanne, France) was applied, and light curing was repeated for 40 seconds.

The DDS was considered the control; the dentin was not sealed directly after preparation.

Temporary Restoration

After dentin preparation, a temporary restoration (Protemp 4, 3M, Seefeld, Germany) was cemented onto the flat dentin surface using a zinc-carboxylate cement (Durelon, 3M). The temporary phase consisted of three weeks of water storage.

Surface Conditioning and Composite Buildup

After three weeks, the temporary restorations were removed with a scaler (H5 Anterior Scaler, Hu-Friedy, Chicago, IL, USA). Next, the surfaces of half of the specimens for each of the four IDS groups were cleaned solely by pumice rubbing (SC-P), while those of the other half of the specimens were cleaned by pumice rubbing and additionally tribochemically silica coated (SC-PS) at a distance of 10 mm following a 45° angulation (2 bar; CoJet sand, SiO_2, 3M). All specimens were subsequently rinsed thoroughly with water and air-dried for 15 seconds, after which a silane coupling agent (ESPE-SIL, 3M) was applied and left to dry (five minutes) according to the method of Ozcan and others.[25] The primer (Optibond FL Primer, Kerr) was then applied onto all specimen surfaces with a light brushing motion for 15 seconds, gently air-dried for 10 seconds, and then suction dried for 15 seconds. A thin layer of heated (40°C) adhesive resin (Optibond FL Adhesive, Kerr) was next applied onto the surface with a light brushing motion for 15 seconds and air-dried for 10 seconds without light curing. An RBC buildup (±6 mm) was subsequently constructed (HFO composite, Micerium, Avegno, Italy) on the prepared surfaces by filling a transparent silicon mold in three to four layers. Caution was taken to avoid air bubbles between the adhesive and RBC layers. The buildup was photopolymerized through the silicon mold using the LED light-curing unit from above and after the last layer it was light-cured for 40 seconds from each of the four sides and from the top; this was repeated after having removed the mold and having applied Glycerin Gel (Johnson & Johnson) at the RBC surface outside.

Aging and μTBS Testing

After one week of storage in 0.5% chloramine T solution at 37°C, microspecimens were cut for μTBS testing. Per experimental group, 24 microspecimens were tested immediately to measure the one-week μTBS, while another set of 24 microspecimens were tested after six months of storage to measure the six-month aged μTBS. The bond strength to dentin was determined using a standardized μTBS protocol.[31] In order to obtain 12 rectangular microspecimen sticks per experimental group (0.9×0.9×8–9 mm), the restored teeth were sectioned perpendicular to the interface using an automated water-cooled precision diamond saw (Accutom-50, Struers, Ballerup, Denmark).[30,40] The dimensions of the sticks were precisely measured by means of a digital caliper (CD-15CPX, Mitutoyo, Kanagawa, Japan) from which the cross-sectional area was calculated (~0.9 mm^2). The microspecimens were fixed to a modified μTBS testing jig[31] using cyanocrylate glue (Model Repair II Blue, Dentsply-Sankin, Tokyo, Japan) and tested in tension mode at a crosshead speed of 1.0 mm/min using an LRX testing machine (Lloyd, Hampshire, UK) equipped with a load cell of 100 N. The bond strength values were calculated in MPa by dividing the imposed force (in N) at the time of fracture by the bonded area (in mm^2). Specimens that failed before actual testing (pretesting failure) were explicitly noted, counted as 0 MPa in further analyses, and thus taken into account for the calculation of the μTBS means.

Failure Pattern Analysis

The failure modes were evaluated using a stereomicroscope (Wild M5A, Wild) at a magnification of up to 50× and classified as follows: failure in dentin, failure at the adhesive-dentin interface, failure in the adhesive resin, failure at the adhesive-composite interface, and failure in the composite. Representative specimens in each group were selected for further ultrastructural characterization using scanning electron microscopy (SEM). The latter specimens were sputter coated using a 3-nm-thick layer of gold (80%) and palladium (20%) (90 seconds, 45 mA; Balzers SCD 030, Balzers, Liechtenstein) prior to

Table 2: *Mean Microtensile Bond Strength (MPa) for the Different Immediate Dentin Sealing (IDS) and Surface-Conditioning (SC) Strategies at One Week and After Six Months of Aging*[a]

	IDS-1L	IDS-2L	IDS-F	DDS
1 wk				
SC-P	29.8 (13.3) aA	26.4 (11.2) aA	29.1 (13.7) aA	30.2 (17.1) aA
SC-PS	35.2 (19.0) aA	39.2 (15.2) aB	28.1 (13.6) aA	16.0 (13.5) bB
6 mo				
SC-P	29.5 (11.6) aA	37.1 (13.9) aA	27.9 (14.3) aA	21.1 (17.0) bA
SC-PS	35.6 (7.3) aA	29.3 (13.7) aA	40.6 (14.9) aA	21.4 (10.3) bA

[a] *Mean (standard deviation); same lowercase letters indicate absence of statistically significant difference in the rows, and same uppercase letters indicate absence of statistically significant difference in the columns (p<.05); IDS-1L, IDS with one layer of adhesive; IDS-2L, IDS with two layers of adhesive; IDS-F, IDS with one layer of adhesive and one layer of flowable RBC; DDS, delayed dentin sealing (control); SC-P, surface conditioning with pumice; SC-PS, SC followed by tribochemical silica coating.*

being examined using a cold field-emission SEM (LEO 440, Leo Electron Microscopy, Cambridge, UK).

Statistical Analysis

All data were analyzed using a statistical software package (SPSS 22, PASW statistics 18.0.3, Quarry Bay, Hong Kong, China). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test for a normal distribution of the data. As the data were normally distributed, the data were divided in two experiments according to aging (one week, six months) and surface conditioning (SC-P, SC-PS). Two-way analysis of variance and *post hoc* testing were applied to verify possible differences among the groups for the parameters of IDS, SC, and aging on μTBS. In all tests, $p < 0.05$ was considered to be statistically significant.

RESULTS

The IDS strategy (F[1, 217.3]=2.265, p=0.087, η^2=0.072) and type of surface conditioning (SC) (F[1, 217.3]=0.061, p=0.806, η^2=0.001) did not produce statistical difference after one week of water aging (Table 2). However, the interaction of IDS and SC strategy was statistically significant (F[3, 217.3]=3.649, p=0.016, η^2=0.111). Hence, the magnitude of the difference between the two SC methods after one week of water aging depends on the IDS strategy that was applied. Silica-coated (SC-PS) specimens from IDS-2L showed the highest mean one-week μTBS. The mean μTBS was significantly higher (Figure 1) when silica coating (SC-PS) was used for this particular IDS strategy (F[1, 217.3]=4.556, p=0.036, η^2=0.049). In contrast, following a DDS strategy, SC-PS achieved significantly lower μTBS than SC-P (F[1, 217.3]=5.630, p=0.020, η^2=0.060).

After six months of water aging, SC did not significantly affect μTBS (F[1, 173.6]=1.099, p=0.297, η^2=0.012), while the IDS strategy significantly influenced μTBS (F[3, 173.6]=5.110, p=0.003, η^2=0.148). The interaction of IDS and SC was not significant (F[3, 173.6]=2.631, p=0.055, η^2=0.082). *Post hoc* tests revealed that regardless of the type of SC, all IDS strategies differed significantly (p=0.001 to p=0.004) from the DDS group but not from each other (Table 2; Figure 2).

Table 2 shows that the aging time (F[1, 200.3]=0.000, p=0.997, η^2=0.000) and IDS strategy

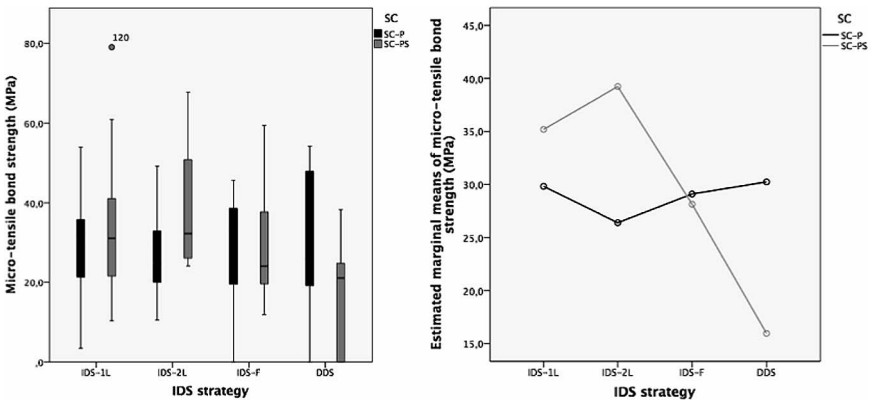

Figure 1. *Box plot of microtensile bond strength (MPa) (left) and estimated marginal means of microtensile bond strength (MPa) (right) for the different immediate dentin sealing (IDS) and surface-conditioning strategies at one week. IDS-1L, IDS with one layer of adhesive; IDS-2L, IDS with two layers of adhesive; IDS-F, IDS with one layer of adhesive and one layer of flowable RBC; DDS, delayed dentin sealing (control); SC-P, surface conditioning with pumice; SC-PS, SC followed by tribochemical silica coating.*

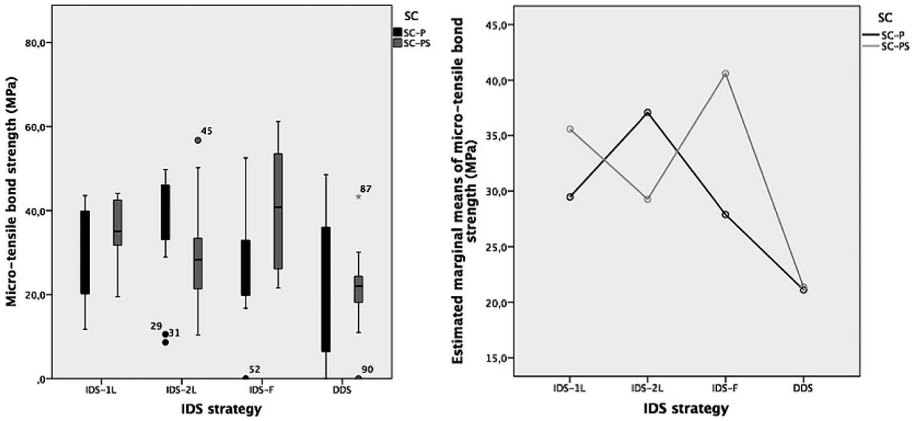

Figure 2. *Box plot of microtensile bond strength (MPa) (left) and estimated marginal means of microtensile bond strength (MPa) (right) for the different immediate dentin sealing (IDS) and surface-conditioning strategies after six months of aging. IDS-1L, IDS with one layer of adhesive; IDS-2L, IDS with two layers of adhesive; IDS-F, IDS with one layer of adhesive and one layer of flowable RBC; DDS, delayed dentin sealing (control); SC-P, surface conditioning with pumice; SC-PS, SC followed by tribochemical silica coating.*

(F[3, 200.3]=0.765, p=0.517, η^2=0.025) did not produce statistical difference for the SC-P groups. There was no interaction of these factors as well (F[3, 200.3]= 1.989, p=0.121, η^2=0.064). However, in general, the SC-P groups demonstrated stable μTBS means in time without significant differences among groups (Figure 3).

For the SC-PS specimens, the aging factor produced no significant difference (F[1, 190.6]=0.538, p=0.465, η^2=0.006). However, there was a highly significant difference in mean μTBS among the IDS strategies (F[3, 190.6]=8.097, p<0.001, η^2=0.216). DDS surfaces conditioned with silica (SC-PS) exhibited the significantly lowest μTBS (p<0.001 for all pairwise comparisons). The aging/IDS strategy interaction was also statistically significant (F[3, 190.6]=2.801, p=0.045, η^2=0.087). Hence, for SC-PS specimens, the magnitude of the difference between the two aging evaluations relied on the strategy of IDS employed. Interestingly, the former was caused mainly by a positive effect of aging on the μTBS means of silica-coated (SC-PS) specimens from the IDS-F group (F[1, 190.6]=4.886, p=0.020, η^2=0.053) (Figure 4), which ultimately led to the highest μTBS reported (Table 2; 40.6±14.9 MPa).

Failure was observed mainly at the adhesive-dentin interface for all the evaluated groups. The fewest failures were seen at the adhesive-composite interface and within dentin or composite (Figure 5). The majority of pretesting failures were related to the DDS groups (Table 3).

DISCUSSION

Glass-ceramic posterior restorations have a good survival rate; however, their prognosis is highly dependent on the strength of the adhesive interface. The weakest link of the interface is the connection of the adhesive to dentin.[10,13,15] In order to increase the adhesive strength of resin-based materials to dentin in indirect restorations, the concept of IDS was introduced. It was shown in an *in vitro* study that ceramic laminate veneers could benefit when large surfaces of dentin were exposed.[15] Freshly exposed dentin is the ideal substrate for dentin bonding.[28,29] How to apply IDS and how to clean or condition the IDS layer during the luting phase has not been studied to date.[32]

After one week of aging, the magnitude of the difference between the two SC methods depended on

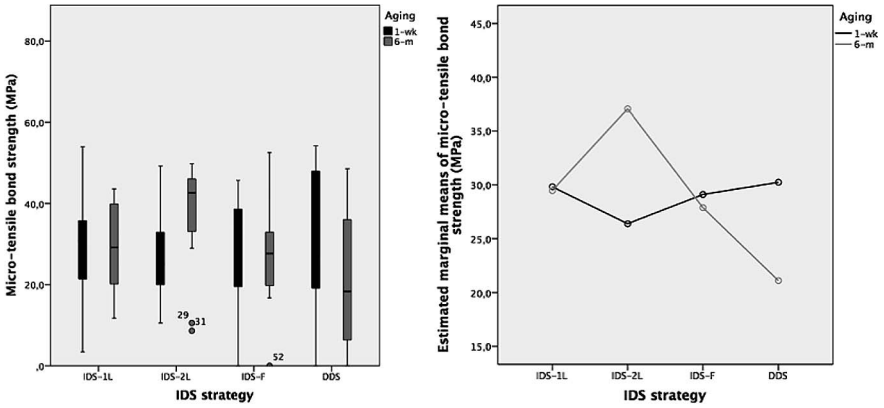

Figure 3. *Box plot of microtensile bond strength (MPa) (left) and estimated marginal means of microtensile bond strength (MPa) (right) for the different immediate dentin sealing (IDS) strategies and two evaluation times (1-wk, at one week; 6-m, after six-month aging) when the surface was conditioned with pumice (SC-P). IDS-1L, IDS with one layer of adhesive; IDS-2L, IDS with two layers of adhesive; IDS-F, IDS with one layer of adhesive and one layer of flowable RBC; DDS, delayed dentin sealing (control).*

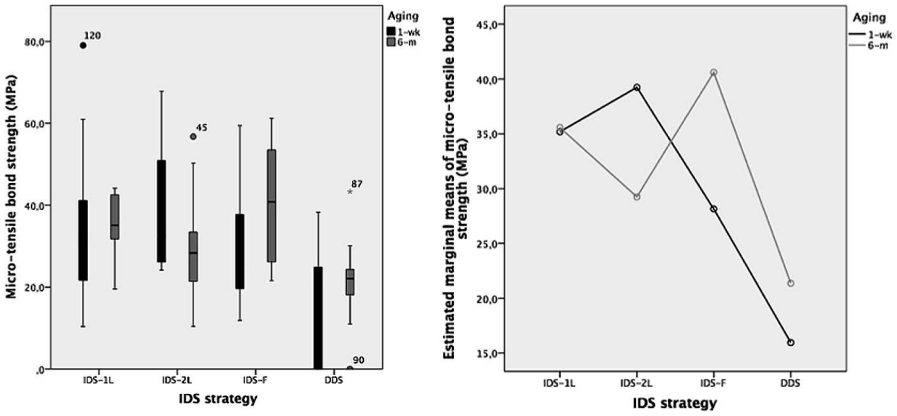

Figure 4. *Box plot of microtensile bond strength (MPa) (left) and estimated marginal means of microtensile bond strength (MPa) (right) for the different immediate dentin sealing (IDS) strategies and two evaluation times (1-wk, at one week; 6-m, after six-month aging) when the surface was conditioned with pumice followed by tribochemical silica-coating (SC-PS). IDS-1L, IDS with one layer of adhesive; IDS-2L, IDS with two layers of adhesive; IDS-F, IDS with one layer of adhesive and one layer of flowable RBC; DDS, delayed dentin sealing (control).*

the type of IDS used. This may be explained by the assumption that with only a thin layer of IDS, tribochemical silica coating, which also involves sandblasting, may largely remove the IDS layer, thereby decreasing bond strength. Hence, a thicker IDS layer and silica coating appeared more favorable. This confirms the observations by Stavridakis and others.[36] They demonstrated the risk of re-exposure of dentin after conditioning the preparation, which may be reduced by using a filled bonding agent like Optibond FL (Kerr). In the present study, the three-step etch-and-rinse adhesive Optibond FL was chosen because this adhesive is known for its high filler load and high mechanical strength resulting in higher μTBS.[10] However, after six months, this particular effect was not observed. All different IDS strategies obtained higher bond strengths than the control group (DDS), independent of the SC method used. Therefore, the adhesive application to dentin directly after preparation is important to achieve higher bond strength. Hashimoto and others concluded that bond strength increased with each adhesive coating up to four layers.[17] Ito and others concluded that simply applying more layers of adhesive could improve the bond strength and the quality of dentin adhesion, especially if the layers were light cured separately.[19] Multiple layers of adhesive resin are thought to create a thicker adhesive layer without affecting the hybrid-layer quality. The increased bond strength results from an improved stress distribution and increased elasticity of the adhesive layer.[4,39] However, others claim that each adhesive has its own ideal thickness and that this should be respected in this multilayering technique.[7] The adhesive system was heated before the application because unpublished data from laboratory studies indicate that it improves μTBS.

In contrast to the results of our study, others have observed that bond strength decreased over time,[10,16] which was attributed to hydrolytic degradation of the adhesive interface.[2,25] Our results demonstrate that by using IDS, the adhesive interface was stable over time; we could not find a

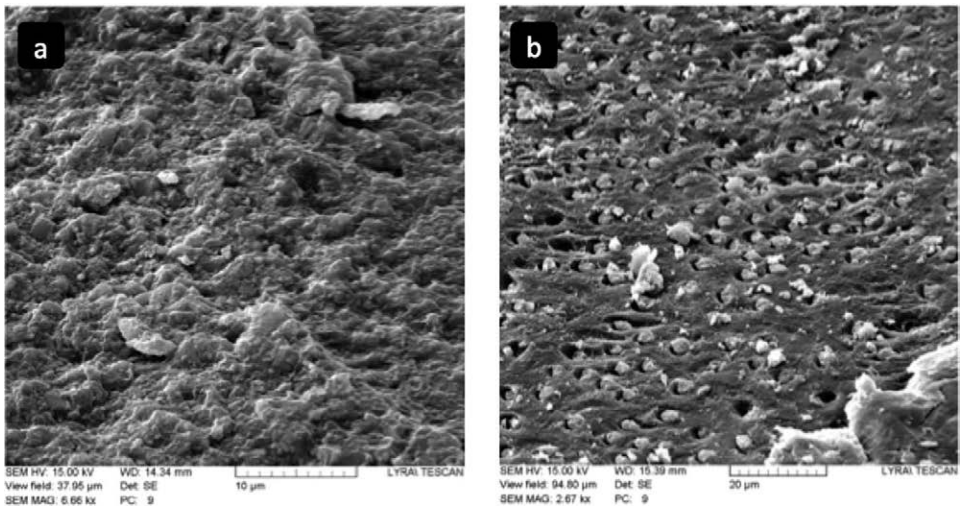

Figure 5. *Scanning electron micrographs of fracture surfaces from representative specimens after microtensile bond strength testing. (a): Adhesive failure at the adhesive-composite interface (IDS-1L, SC-PS, 6-m). (b): Adhesive failure at the adhesive-dentin interface (DDS, SC-PS, 6-m). Note the exposed dentin tubuli. IDS, immediate dentin sealing; IDS-1L, IDS with one layer of adhesive; IDS-2L, IDS with two layers of adhesive; IDS-F, IDS with one layer of adhesive and one layer of flowable RBC; DDS, delayed dentin sealing (control); 1-wk, at one week; 6-m, after six-month aging.*

Table 3: *Overview of Pretesting Failures (ptf), Total Number of Specimens (n), and Failure Analysis (%)*[a]

	ptf	n	S	I	B	B/C	C
1 wk							
IDS-1L + SC-P	0	12	11	67	22		
IDS-1L + SC-PS	0	12		17	17		67
IDS-2L + SC-P	0	12		67			33
IDS-2L + SC-PS	0	12	50	33			17
IDS-F + SC-P	1	12		63	25	13	
IDS-F + SC-PS	0	12	11	78			11
DDS + SC-P	1	12		100			
DDS + SC-PS	4	12		100			
6 mo							
IDS-1L + SC-P	0	12	9	91			
IDS-1L + SC-PS	0	12		80		20	
IDS-2L + SC-P	0	12		76			25
IDS-2L + SC-PS	0	12	25	75			
IDS-F + SC-P	1	12		33	67		
IDS-F + SC-PS	0	12		67			33
DDS + SC-P	3	12		80	20		
DDS + SC-PS	1	12		50	50		

[a] *IDS, immediate dentin sealing; IDS-1L, IDS with one layer of adhesive; IDS-2L, IDS with two layers of adhesive; IDS-F, IDS with one layer of adhesive and one layer of flowable RBC; DDS, delayed dentin sealing with no layer of adhesive (control group); SC, surface conditioning; SC-P, surface conditioning with pumice rubbing; SC-PC, surface conditioning with pumice followed by tribochemical silica coating; 1-wk, immediate; 6-m, aged; ptf, pretesting failure; S, failure in dentin; I, failure at the adhesive-dentin interface; B, failure in adhesive; B/C, failure at the adhesive-composite interface; C, failure in composite.*

significantly different effect between the one-week and six-month water storage data. Further investigation is needed to find out if this could be due to an improved resin impregnation associated with an IDS application. According to Magne and others, good bond strength of the definitive restoration to the sealed dentinal surface can be achieved even up to an extended provisionalization phase of 12 weeks.[23] In this study, the provisionalization phase was three weeks, which, according to Magne and others, could not have affected bond strength.[23]

When surfaces were cleaned using pumice, a stable μTBS over time was recorded without a significant difference among the IDS strategies. Surfaces conditioned with tribochemical silica coating demonstrated a more positive effect after six months of aging than the solely pumice-rubbed surfaces. A higher mean bond strength was obtained with a thicker IDS layer compared to a DDS strategy. High mean μTBS values and small standard deviations were seen in the IDS-1L group, when specimens were silica coated, after one week (35.2±19.0 MPa) and six months (35.6±7.3 MPa) of aging. Others attributed the improved bond strength recorded using tribochemical silica coating to the additional chemical bonding of the applied silane coupling agent to the silica-coated surface.[1,33,37]

Most failures in the one-week and six-month aged groups were seen at the adhesive-dentin interface; it might be interesting to see whether there would be more differences in bond strength after a longer period of aging. Pretesting failures were seen mainly in the DDS groups, probably due to water uptake of the dentin. In the DDS group, no adhesive interface was created (directly after preparation), while this adhesive interface was necessary to prevent water uptake from the tooth (in the temporary crown phase).[18] A disadvantage of the μTBS is not only that the actual "bond" strength measured but also that the strength of the complete assembly, including dentin and composite,[9] possibly results in a higher strength that surpasses the interfacial strength. Yet μTBS is generally accepted as one of the most valid bond strength tests.[35] According to a review by Qanungo and others[32] and based on the results of this study, significant differences have been demonstrated between IDS and DDS, this being more in favor of IDS.

CONCLUSIONS

From this study, the following can be concluded:

1. The μTBS recorded for specimens prepared with any type of the three IDS strategies investigated was higher compared to DDS.
2. Cleaning with pumice only or with additional tribochemical silica coating did not affect μTBS. When using a silica-coating technique, a thick IDS layer is recommended.
3. A one-layer IDS and surface conditioning involving pumice rubbing with additional tribochemical silica coating appears to be an effective, consistent, durable, and relatively less time consuming IDS procedure.

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Regulatory Statement

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

guidelines and policies of Rijksuniversity Groningen in the Netherlands.

Conflict of Interest

The authors did not have any commercial interest in any of the materials used in this study.

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Effect of Ferrule Thickness on Fracture Resistance of Teeth Restored With a Glass Fiber Post or Cast Post

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Clinical Relevance

A tooth without a ferrule presented more favorable failures than with a 1-mm-thick ferrule when restored with a cast post and core, despite an increased fracture resistance. The findings support the use of a glass fiber post.

SUMMARY

Purpose: To investigate the influence of ferrule thickness on fracture resistance after mechanical cycling of teeth restored with different intracanal posts.

Methods and Materials: One hundred twenty bovine incisor teeth were randomized into six study groups, based on the intracanal post used (fiber post or cast post and core) and the presence and thickness of a ferrule (without ferrule, presence of 0.5-mm or 1-mm-thick ferrule, retaining unaltered 2-mm ferrule height). The root posts and the metal crowns were cemented using an adhesive cement. The samples were subjected to mechanical cycling (at 37°C, 45°, 130 N, 2.2 Hz, and 2×10^6 pulses). Afterward, they were subjected to a fracture load test at a speed of 0.5 mm/min and on a 45° slope until failure occurred. The failures were classified as favorable or unfavorable. The fracture resistance data were analyzed with two-way analysis of variance and Tukey test. The chi-square test was used to analyze the pattern of failure.

Results: When considering the cast post and core, the 1-mm ferrule thickness group pre-

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sented a higher resistance to fracture than did the group in which a ferrule was not used (p=0.001). When using a glass fiber post, the groups showed no differences in fracture resistance. Overall, 96.7% of the specimens survived the mechanical cycling. Of the fractures, 58.6% of the fractures were unfavorable, while 41.6% were favorable.

Conclusions: A thicker ferrule statistically increased the fracture resistance only for cast post and core when it was at least 1 mm thick, despite causing more unfavorable failures. Thus, ferrule thickness should be considered when choosing different intracanal posts, to reduce the occurrence of unfavorable failures. In the absence of a ferrule, the use of a cast post and core presents more favorable failures, and in the presence of a 1-mm-thick ferrule, the use of a glass fiber post seems to be the best clinical decision.

INTRODUCTION

The prognosis of endodontically treated teeth depends on several factors, such as adequate coronal reconstruction,[1] tooth position in the dental arch, type of final restoration, post length and thickness, and the presence of a ferrule.[2] A ferrule is composed of parallel walls of dentin from the crown's margin extending coronally to the fractured part of the tooth (see Figure 1).[2,3] Fabricating a crown around the remaining structures and generating a ferrule effect[2-4] may reduce intraradicular stress and thus the incidence of fractures.[5]

The clinical outcome is significantly influenced by the amount of residual coronal dentin,[6] and the existing literature extensively describes the importance of having adequate ferrule height.[7] Studies have demonstrated that a minimum ferrule height of 1.5-2 mm shows improvement in the longevity of endodontically treated teeth restored with post and core[2,7,8] and also provides better fracture resistance.[7,9]

The influence of ferrule thickness on clinical outcome is also a topic that needs to be explored further.[7] The amount of residual axial tooth structure to be significant in fracture resistance has been reported in the literature[7,10,11]; however, some reports have excluded the width of shoulder preparation and crown margin as significant factors. The shoulder preparation could compromise the thickness of the buccal dentinal wall when esthetics require more invasive preparations at the margin or

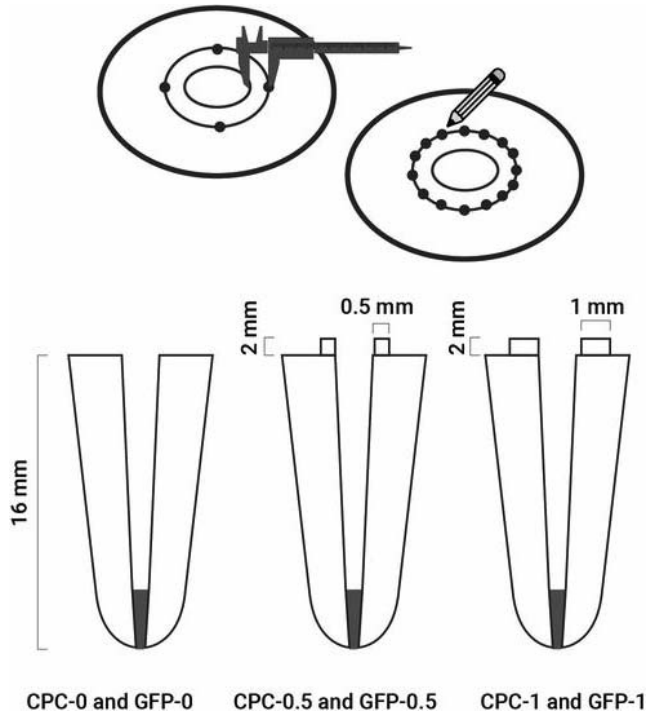

Figure 1. *Schematic diagram of the marking of the ferrule thickness and experimental groups.*

because of existing carious lesions.[7] For instance, ceramic restorations commonly require that the remaining dentin thickness at the margins of the preparations are reduced by at least 1.5 mm to achieve desirable esthetics.[7,12]

Although it has been accepted clinically that a ferrule thickness of 1 mm is considered very thin,[7] there are only a few studies in the literature reporting the effect of remaining coronal thickness on the mechanical behavior of endodontically treated teeth.[13,14] Tjan and Whang[13] reported that there was no significant difference among the groups that had remaining buccal dentin of varying thickness of 1 mm, 1 mm with a 60° bevel, 2 mm, and 3 mm. However, a dentin thickness of 1 mm resulted in a higher incidence of failure due to fracture rather than cement failure.[13] In addition, Joseph and Ramachandran[14] studied the effectiveness of incorporating the thickness of coronal dentin and concluded that fracture resistance increased in the case of 2-mm-thick remaining dentin.[14] On the other hand, there are no studies in the literature evaluating the effect of 0.5-mm ferrule thickness on resistance to fracture.[7]

Thus, the objective of this research was to evaluate the influence of the remaining coronal thickness (without ferrule, 0.5-mm thickness, and 1-mm

Table 1: *Study Design*

Study Factors		Group Code
Kind of Root Posts	**Thickness of Ferrule**	
Glass fiber post	No ferrule	GFP-0
	Ferrule with a height of 2 mm and a thickness of 0.5 mm	GFP-0.5
	Ferrule with a height of 2 mm and a thickness of 1 mm	GFP-1
Cast post and core	No ferrule	CPC-0
	Ferrule with a height of 2 mm and a thickness of 0.5 mm	CPC-0.5
	Ferrule with a height of 2 mm and a thickness of 1 mm	CPC-1

thickness) on the resistance to fracture of endodontically treated teeth with a glass-fiber post or cast post and core. The null hypothesis was that ferrule thickness has no influence on the fracture resistance of endodontically treated teeth restored with different intracanal posts after mechanical cycling and fracture load test.

METHODS AND MATERIALS

Selection of Specimens

The number of teeth to be used in the present research was determined by performing a sample calculation with the OpenEpi 3.01 program,[15] with the parameters used previously in a study by Wandscher and others.[16] The power of the study was defined as 80%, with a level of significance of 0.05, identifying the need for 15 teeth per group. However, because of the variability of bovine teeth, radicular anatomy, and possible variability in the preparation of the ferrule, 20 teeth were allocated to each group.

One hundred twenty bovine incisor teeth were selected and analyzed for possible fractures, cracks, and fissures, with the aid of a loupe (4× magnifying, EyeMag Pro S, Carl Zeiss, Gottingen, Germany). Afterward, the selected teeth were randomized through the website random.org into six groups (n=20 in each) based on the post type (glass fiber post [GFP] or cast post and core [CPC]) and features of the ferrule (Table 1).

Thereafter, the coronal portion of each tooth was sectioned at a distance of 16 mm (in the absence of a ferrule, CPC-0 and GFP-0) or 18 mm (in the presence of a 2-mm-high ferrule, with varying thickness, CPC-0.5, CPC-1, GFP-0.5, and GFP-1) from the root apex, resulting in a standard height. All procedures were performed by two trained researchers.

To avoid differences in tooth size among the groups, the mesiodistal and vestibular-lingual dimensions of the teeth were measured with the aid of digital calipers (Starrett 727, Starrett, Itu, São Paulo, Brazil), and the measurements were tabulated. The data were verified to be normally distributed. Subsequently, a one-way analysis of variance (ANOVA) was performed to verify differences in the measured dimensions between the groups. No statistically significant difference (α=0.05) in the dimensions of the teeth could be detected.

Periodontal Ligament Confection and Endodontic Treatment

To simulate the periodontal ligament and the biological space, the roots were coated with a layer of wax (Lysanda, São Paulo, Brazil) of 0.3-mm thickness (measured by digital calipers; Starrett 727, Itu). For this purpose, the wax was liquefied in a container at a standard temperature of 70°C, and each tooth was placed inside up to 3 mm below the most coronal portion of the root. Then, each tooth was embedded in a polyvinyl chloride cylinder (height of 20 mm and diameter of 25 mm) with self-cured acrylic resin (VIPI Flash, VIPI, Pirassununga, São Paulo, Brazil) up to 3 mm below the most coronal portion of the root, simulating the biologic space. Afterward, the tooth was removed from the acrylic resin and the wax was removed, creating a space corresponding to the periodontal ligament. Subsequently, the impression material was manipulated as recommended by the manufacturer and inserted into the artificial alveolus. The tooth was placed into its respective alveolus, and excess impression material was removed; thus, the elastomeric material (Impregum F, 3M-ESPE, Seefeld, Germany) mimicked the periodontal ligament.[17]

Afterward, the root was prepared using the step-back technique, using second- and third-series endodontic files (Dentsply-Maillefer, Ballaigues, Switzerland) and Nos. 3, 4, and 5 Gates-Glidden burs (Dentsply-Maillefer). The specimens were filled with AH plus sealer (Dentsply-Maillefer), and the root canals were obturated with gutta-percha cones (Dentsply-Maillefer). The compaction technique used was cold lateral condensation with a force of 2000*g* standardized through a digital scale.[18] The specimens were stored in deionized water at 37°C for 24 hours.

Intracanal Preparation for Post Seat

A post space (cementation length) of 12 mm was prepared for the groups without ferrule (CPC-0,

GFP-0), and 14 mm preparation was used for the groups with ferrule (GFP-0.5, GFP-1, CPC-0.5, CPC-1). For the GFP-0, GFP-0.5, and GFP-1 groups, the preparation was initially performed by No. 4 Largo burs (Dentsply Maillefer) and finished with standardized drills of the Whitepost DC No. 2 fiberglass post system (FGM, Joinville, Brazil). For the CPC-0, CPC-0.5, and CPC-1 groups, the post space was prepared using Nos. 3, 4, and 5 Largo burs (Dentsply Maillefer).

Preparation of Ferrule

The ferrules in the GFP-0.5, GFP-1, CPC-0.5, and CPC-1 groups were manually prepared with a diamond bur (No. 3216, KG Sorensen, Barueri, Brazil) using a high-speed hand piece (Extra Torque 605C; Kavo do Brasil, Joinville, Brazil) with water spray cooling, resulting in the corresponding thickness of each group and standard height of 2 mm. For this, the thickness and height to be removed were marked with graphite with the aid of a digital caliper (Starrett 727, Itu; Figure 1).

Production of the Cast Post and Cores

The standards for the cast post and core were obtained by molding the root canals with chemically activated acrylic resin Bosworth Trim Plus (Bosworth Company, Skokie, IL, USA) and prefabricated plastic posts (Pinjet, Ângelus, Londrina, Paraná, Brazil). For preparation of the coronal part of the core, acetic matrices were used.

Resin patterns were handed over to a commercial laboratory for casting. Next, the cast post and cores were evaluated for adaptation.

Cementation of the Posts and Crowns

Prior to post cementation, the intracanal surfaces of the cast post and cores were air-abraded with aluminum oxide particles (110 μm, pressure 2.8 bars, 10-mm distance and 15 seconds; Blue, São José do Rio Preto, Brazil).

For the glass fiber post groups and cast post and core groups, 7 mm of the coronal portion of the post was retained. All surfaces of each glass fiber post were cleaned with 70% alcohol. Next, a silane coupling agent (Prosil, FGM, Joinville, Santa Catarina, Brazil) was applied to each post, and the solvent was allowed to evaporate for five minutes.

All root posts were cemented using the same procedures: the root canal and the coronal portions were prepared using 37% phosphoric acid (Condac 37, FGM), and the adhesive Ambar (FGM) was applied according to the manufacturer's guidelines. Finally, the posts were cemented with dual-cure resin cement (Allcem, FGM), which was also manipulated as recommended by the manufacturer.

The cores (for glass fiber post samples) were fabricated with a composite resin (Opallis, FGM) with acetic matrix (identical to that used for the CPC-0 groups). The matrix was filled with increments of composite resin and adapted over the coronal portion of the post. Afterward, the matrices were sectioned and the composite resin was photo activated (1200 mW/cm^2; Radiical, SDI, Victoria, Australia) on each side of the tooth for 10 seconds.

For all groups, metallic full crowns (Ni-Cr alloy; Wirona light, Bego, Goldschlagerei, Germany) were prepared with standardized shape and dimensions, according to the anatomy of a maxillary canine. After that, the crowns were evaluated and air-abraded with aluminum oxide (110 μm, pressure 2.8 bars, 10-mm distance, and 15 seconds).

Before the crowns were cemented, they were cleaned with absolute alcohol. The dentin and core (composite and metal) surfaces were etched with 37% phosphoric acid for 15 seconds, followed by rinsing with air-water spray and drying with absorbent paper, and the adhesive Ambar (FGM) was applied according to the manufacturer's guidelines. The full-metal crowns were cemented with a dual-cure resin cement (Allcem, FGM), according to the manufacturer's guidelines. Next, a 5-kg load was applied on each metal crown by means of a static press, during cementation. Excess cement was removed after three minutes, and photo-activation was carried out (1200 mW/cm^2, Radiical, SDI) on each side of the tooth for 10 seconds. The samples were stored for 24 hours before testing.

Mechanical Cycling

The specimens were subjected to mechanical cycling (Erios ER 3000, São Paulo, Brazil) for the aging, with the following protocol: 2.2-Hz frequency, 2 million load pulses from 0 N to 130 N, immersion in water at 37°C temperature, and piston at a 45° angle with respect to the long axis of the root and at 2 mm distance from the lingual incisal edge. Thus, in this trial, approximately two years of clinical service was simulated, since Wiskott and others[19] determined that 1 million cycles correspond to one year of service.

Fracture Load Test

After mechanical cycling, the specimens were analyzed for the presence of fractures, and those that did

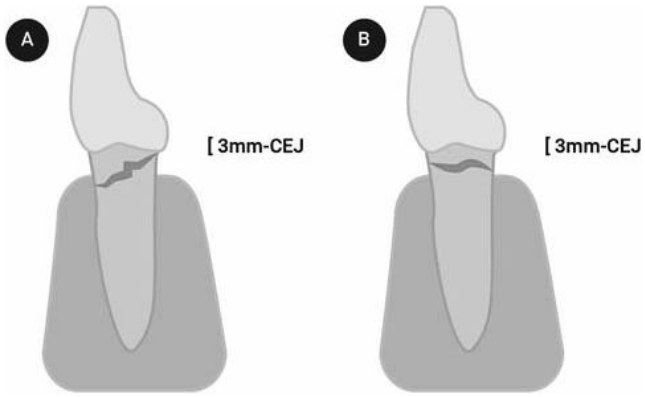

Figure 2. *Schematic diagram of failures. (A) Failure unfavorable. (B) Failure favorable.*

not present with cracks were subjected to the fracture load test in a universal test machine (DL 2000, Emic, São José dos Pinhais, Brazil). Each sample was positioned on a fixed metal device and aligned at a 45° angle, under 0.5 mm/min until failure occurred. The cylindrical metallic tip (diameter 0.8 mm) attached to the load cell (1000 kN) was applied as a lingual load (2 mm from the lingual incisal edge). A load point at which the force resulted in root fracture, post curvature, or core and post displacement was defined as the threshold of failure.

Failure Analysis

The roots were stained superficially with hydrographic pens (Blue overhead marker, Faber-Castell, São Carlos, Brazil), after the fracture load test. The excess ink was then removed with cotton and 70% alcohol, and the specimens were visualized with a stereomicroscope at 10× magnification (Stereomicroscope Discovery V20; Carl Zeiss, Germany). The failures were classified as favorable (ie, above the 3-mm limit of the acrylic resin, up to the limit of the simulated cemento-enamel junction [CEJ]) and unfavorable (ie, below the aforementioned limit, below the CEJ; Figure 2). Fractured specimens were transversely sectioned in a cutting machine (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) with a diamond saw, making it possible to inspect the crack and its features.

Data Analysis

Using the Shapiro-Wilk test, the fracture load data were analyzed for their distribution, while their homogeneity was analyzed using the Levene test. They were found to have a homogeneous and normal distribution ($p>0.05$). Then, the fracture load data were submitted to two-way ANOVA and Tukey tests ($\alpha=0.05$). In addition, the chi-square test was used to analyze the association between the different patterns of failure and the different groups.

RESULTS

Mechanical Cycling

In total, 96.7% of the specimens survived mechanical cycling. In the GFP-0 group, two failures occurred, one being favorable, presenting a crack at the vestibular region and detachment of the crown from the lingual region, and one unfavorable, with cracks at the proximal and lingual regions, besides displacement of the crown from the lingual region. The unfavorable fracture occurred in the GFP-0.5 group with cracks at the proximal region and displacement of the crown from the lingual region. In the CPC-0.5 group, only an adhesive failure was observed in the lingual region. In the CPC-0, GFP-1, and CPC-1 groups, there were no failures during cycling (Table 2).

Fracture Load

Table 3 shows the fracture load data (N). The Tukey test showed a significant difference between the groups CPC-0 and CPC-1 ($p=0.001$). The other groups did not demonstrate significant differences.

Failure Analysis

Table 2 enumerates the failures that occurred during the fracture load test and mechanical cycling, in addition to the location of fracture. Of the fractures, 58.6% of the fractures were unfavorable, while 41.6% were favorable. In the groups that used a glass fiber post, the GFP-1 group showed the most favorable failures (60.0%), while among the groups that used the cast post and core, the CPC-0 group presented more favorable failures (80.0%). After the fracture load test, the surface that presented the most cracks in the radicular third was the distal surface, followed by the mesial. Furthermore, displacement of the lingual portion of the crown occurred in 82% of the specimens.

The pattern of failure was analyzed using the chi-square test (Table 4). When comparing groups that used the same type of post but different thicknesses of the ferrule, it was observed that in the glass fiber post groups, only the GFP-0.5 and GPF-1 groups showed a statistically significant difference between them ($p=0.01$); among the groups that used a cast post and core, there was a statistical difference between the CPC-0 and CPC-0.5 groups ($p=0.001$) and between the CPC-0 and CPC-1 ($p=0.001$) groups. On the other hand, when comparing groups

Table 2: *Qualitative Evaluation of Failures After Mechanical Cycling and Fracture Load Test*

	Study Group, n (%)						
	GFP-0	**GFP-0.5**	**GFP-1**	**CPC-0**	**CPC-0.5**	**CPC-1**	**Total**
Failures during mechanical cycling							
Pattern of failure							
Favorable	1	—	—	—	1	—	2
Unfavorable	1	1	—	—	—	—	2
Failure place							
Crown displacement (lingual)	2	1	—	—	—	—	3
Mesial crack	1	1	—	—	—	—	3
Buccal crack	1	—	—	—	—	—	1
Distal crack	1	1	—	—	—	—	2
Lingual crack	1	—	—	—	—	—	1
Fracture in the post	—	—	—	—	—	—	—
Crown, core, post pull out	—	—	—	—	1	—	1
Failures after fracture load							
Pattern of failure							
Favorable	8 (44.4)	4 (21.1)	12 (60.0)	16 (80.0)	4 (21.1)	4 (20.0)	48 (41.4)
Unfavorable	10 (55.6)	15 (78.9)	8 (40.0)	4 (20.0)	15 (78.9)	16 (80.0)	68 (58.6)
Failure place							
Crown displacement (lingual)	17	15	19	14	16	17	98
Mesial crack	11	16	15	10	15	16	83
Buccal crack	8	3	10	9	8	15	53
Distal crack	13	16	16	6	16	18	85
Lingual crack	1	—	—	1	—	3	5
Fracture in the post	—	—	—		—	1	1
Crown, core, post pull out	2	1	—	5	3	3	14
Failure mode							
Mesiodistal	10	16	15	6	11	16	
Buccolingual	—	—	—	—	—	1	

with different types of posts but the same ferrule thickness, the groups with a 0.5-mm-thick ferrule presented no differences among them, while the other groups presented differences.

DISCUSSION

The present study showed that remaining coronal thickness affected the fracture resistance of endodontically treated teeth that were restored using a cast post and core. Thus, the null hypothesis was rejected.

The mechanical cycling of the specimens was performed by simulating an aging condition close to a real-life situation. Applying 2 million cycles simulated approximately two years of clinical service.[19] We found that 96.7% of the specimens survived the mechanical cycling. Two failures in the GFP-0 group and one in the GFP-0.5 group were reported on analysis of the failures that occurred following mechanical cycling in the groups that used glass fiber posts. However, the group with the highest ferrule thickness (GFP-1) exhibited no failure, which corroborates that the greater the

Table 3: *Mean (± Standard Deviation) of the Results of Fracture Load (N) Test and Tukey's Test*[a]

Post	**Thickness of Ferrule**		
	Without Ferrule	**0.5 mm**	**1 mm**
Glass fiber post	352.66 ± 219.02 aA	462.39 ± 272.65 aA	474.30 ± 219.67 aA
Cast post and core	339.04 ± 153.78 aB	483.69 ± 342.77 aAB	575.72 ± 214.34 aA

[a] *Uppercase letters compare groups with the same intracanal post but different ferrule thickness (rows). Lowercase letters compare groups with the same ferrule thickness but different intracanal post (column).*

Table 4: *Association Between Groups and Pattern of Failures, Chi-Square Test*[a]

Pattern of Failure	Thickness of Ferrule, %		
	Without Ferrule	0.5 mm	1 mm
Glass fiber post	ABb	Ba	Aa
Favorable	44.44	20	60
Unfavorable	55.56	80	40
Cast post and core	Aa	Ba	Bb
Favorable	80	21.05	20
Unfavorable	20	78.95	80

[a] *Uppercase letters compare groups with the same intracanal post but different ferrule thickness. Lowercase letters compare groups with the same ferrule thickness but different intracanal post.*

ferrule thickness, the higher the chance of longer tooth survival without failure.

The CPC-0 and CPC-1 groups presented a significant difference in the fracture resistance test, since the CPC-1 group had a higher value of fracture resistance, followed by the CPC-0.5 group and, finally, the CPC-0 group. However, there was no significant difference when the thickness varied by only 0.5 mm (CPC-0 for CPC-0.5; CPC-0.5 for CPC-1; Table 3). This can be explained by the small increase in ferrule thickness (0.5 mm) because clinically, the coronal walls are considered to be very thin when they are less than 1-mm thick.[7] Furthermore, the number of unfavorable failures also increased when the ferrule thickness reached 0.5 mm or 1 mm (Table 2). The possible reason for this was an increase in the fracture resistance with a thicker ferrule. Moreover, an increase in unfavorable failures can also be explained by the high modulus of elasticity of nickel-chromium (Ni-Cr) that can concentrate stresses through the post and core into the walls of the root canals.[20,21]

On analysis of the groups that used glass fiber posts, no increase in fracture resistance was found with increasing ferrule thickness (Table 3). This may be due to better stress distribution by the glass fiber post as compared with the cast post and core.[22] In accordance with these results, in a classic study by Sorensen and Engelman,[23] it was shown that the axial width of the tooth at the crown margin did not significantly increase the fracture resistance or alter the failure threshold. In addition, a clinical study by Mancebo and others[24] demonstrated that endodontically treated teeth with a ferrule, that were restored with glass fiber posts, showed better clinical survival after 3 years of clinical service compared with nonferrule teeth. In agreement with Macebo and others,[24] in this study, the failures were more favorable in the endodontically treated teeth restored with a glass fiber post and 1-mm-thick ferrules when compared with teeth treated endodontically and restored with glass fiber posts without ferrules.

The chi-square test (Table 4), using the proportions of failure pattern in each group, was performed to determine whether there was a difference in the failure mode between different types of posts and ferrule thicknesses. When the specimen had no ferrule—in the GFP-0 (44.4% favorable failures) and CPC-0 groups (80.0% favorable failures; $p=0.02$)—the cast post and core could be used because there were more favorable failures. When the specimen had a 0.5-mm-thick ferrule, either of the post types could be used because there was no statistically significant difference between their failure patterns ($p=0.77$). In contrast, with a 1-mm-thick ferrule, a fiber post (60.0% favorable failures) will be more suitable than a cast post and core (20.0% favorable failures; $p=0.01$).

According to Santos and others,[20] in order to choose a suitable technique to restore endodontically treated maxillary incisor teeth, we must take into account the amount of dental structure remaining and the esthetic and functional considerations. Furthermore, there is evidence in the literature supporting the clinical choice of glass fiber post when there is more remnant coronal structure and cast post and core when there is none.[22,25,26] In this study, we observed that the CPC-0 subgroup had a higher percentage of favorable failures than the GFP-0 subgroup, and the GFP-1 subgroup had more favorable failures than the CPC-1 subgroup (Table 4), which agree with the results reported in the literature. Figure 1 shows that the teeth without any coronal remnant could probably suffer from more bending than those with some remnant. It is stated that the fiber post concentrated stresses at the cervical level[27]; hence, the stress distribution to dentin in this region is higher. However, when the ferrule effect exists, the fiber post presents with less tendency to bend.

Recently, Wandscher and others analyzed flared roots restored with different types of posts that fractured after cyclic and static loading (demonstrated by means of fractographic analysis) and found that the final fracture was a consequence of tensile, compression, and shear stresses.[16] Taking into consideration the interpretation of the fractured surface, failure analysis revealed the direction of crack propagation, thus recognizing the origin or cause of failure.[28] The origin of the fracture may be a location, a specific flaw, or an irregularity. The nature of loading (tensile, bending, shear, torsion, and fatigue), microstructure of the material, envi-

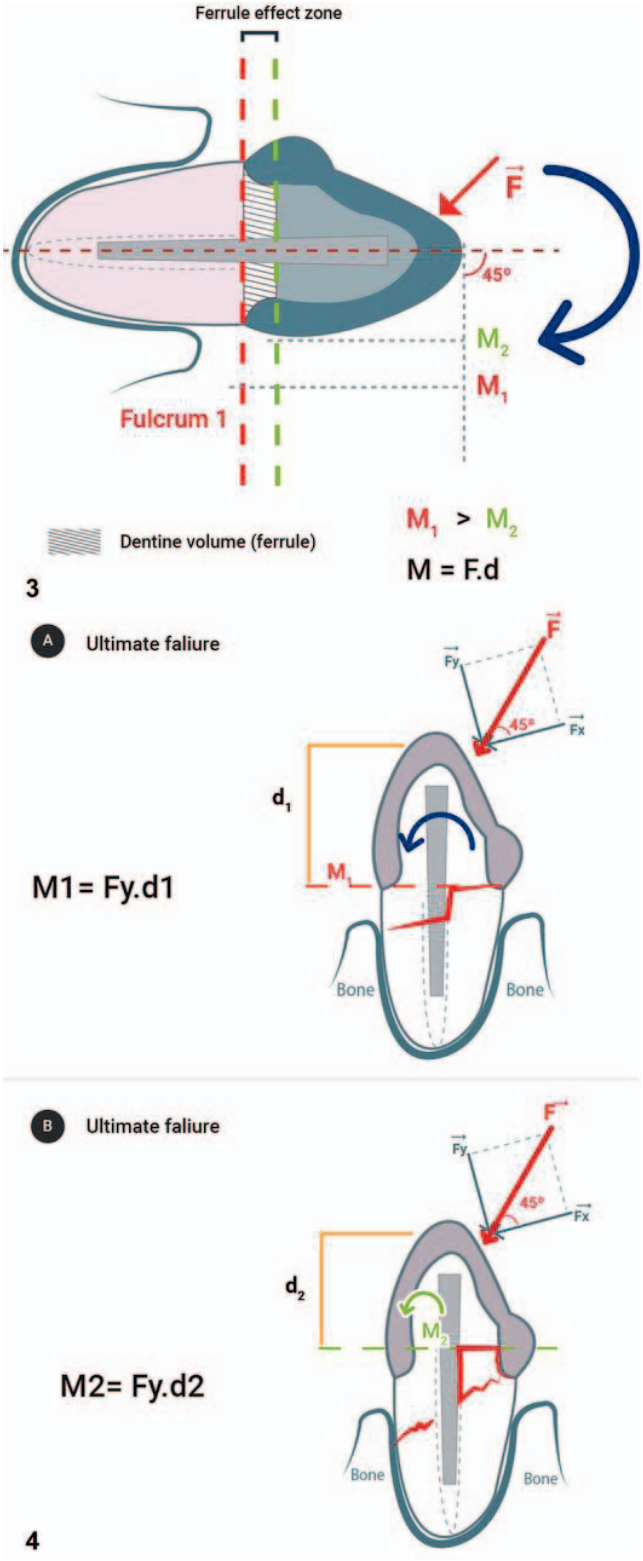

Figure 3. *Schematic representation of a restored tooth with a post subjected to an oblique force. $\vec{F}$: force exerted on the specimen (45°); fulcrum 1: fulcrum formed when there is no ferrule present in the specimen (GFP-0 and CPC-0 groups); fulcrum 2: fulcrum formed when there is presence of ferrule in the specimen (groups GFP-0.5, GFP-1, CPC-0.5 and CPC-1). M: bending moment (measured by multiplying the force applied by the distance between the point of application of the load and the fulcrum line); F: applied force; D: distance from the point of application of the load to the fulcrum line; M1: bending moment referring to the fulcrum 1; M2: bending moment relative to fulcrum 2; dentine volume: volume of coronary remaining present in specimens with the presence of ferrule.*

ronment factors, and stress concentrators indicate the appearance of marks at the location of the origin of the failure.[29] A few studies of endodontically treated teeth restored with posts present a detailed analysis of the fractured specimens. Therefore, only a few studies have evaluated in detail the fractured surfaces of teeth with remaining coronal structure (the ferrule effect). However, those results strongly suggest that the remaining coronal thickness might influence the stress distribution on the tooth/post/crown, consequently affecting its fracture resistance and the mode of fracture.

In relation to the failure pattern, it is observed that the roots fractured at similar rates (Table 2) as those in the study by Wandscher and others,[16] presenting mesiodistal cracks independent of the type of post used. This feature is a consequence of the loading mode of the specimens (45°). Biomechanical studies demonstrated that restored teeth subjected to oblique loads suffered tensile (lingual surface) and compression (buccal surface) forces.[16,30,31] These tensions are maximal in the external portions (lingual and vestibular) and minimal in the center of the restorative set (root canal). The authors stated that a sequence of events led to the final fracture, such as shear stresses on the post-dentin adhesive interface that caused decementation of the assembly (crown displacement); thus, the post becomes loose in the canal, no longer acting as a unique structure and breaking on the buccal wall due to higher compressive stress.

In the current study, it was observed that the presence of the ferrule effect on a restored tooth with a post altered the arrangement of the forces acting on the restored tooth (without ferrule effect vs with ferrule effect groups). A tooth with a cervical ferrule presents a coronal displacement of the fulcrum line, decreasing the effect of flexion (bending moment)[32] and protecting the specimen (Figure 3). This fact explains why teeth with a ferrule effect present a higher loading fracture.

Figure 4. *(A): Specimens of the glass fiber post group or cast post and core group without a ferrule. (B): Specimens of the glass fiber post group or cast post and core group with a ferrule. Schematic drawing of the ultimate fracture without and with ferrule specimens ($\vec{F}$: force exerted on the specimen (45°); $\vec{F}_y$: vertical component of F; $\vec{F}_x$: horizontal component of F; d1: distance from the point of application of the load to the specimen; M1: bending moment of the specimen, red line).*

Comparing the groups with and without a ferrule effect, some differences in the failure pattern were observed (Figure 4). The nonferrule specimen presented with an adhesive failure between the crown and root (lingual surface), followed by a crack into the canal that finished on the buccal root dentin (Figure 4A). In contrast, the ferruled specimen presented with the same adhesive failure (crown/root) with a crack starting on the prosthetic shoulder into the canal (Figure 4B). The presence of the ferrule between the post and crown created a lever arm due to the bending movement (Figure 4). Therefore, it is possible to conclude that this feature protected the restored assembly. In addition, despite a nonstatistical difference between the groups, we concluded that the greater the thickness of the ferrule, the greater was the lever arm and fracture load. Moreover, some studies showed that the presence of a ferrule of 1.5 to 2 mm height was more important for fracture resistance than the post type or post design.[9,33,34]

Studies evaluating the fracture load of endodontically treated teeth restored with posts and tested *in vitro* presented higher values of fracture than the probability of the load to failure clinically; consequently a large standard deviation was obtained (Table 2).[31,35] Thus, new studies applying fatigue tests (such as staircase or step-stress approaches) should be conducted to predict the fatigue behavior of the restored roots. Furthermore, other factors such as loading direction, pH alterations, humidity, and temperature were difficult to simulate in *in vitro* tests. Further studies should be performed to develop methods or techniques that allow a detailed analysis of the fractured surface of endodontically treated teeth restored with posts.

CONCLUSIONS

- The fracture resistance increased statistically only when a ferrule thickness of 1 mm was present for the cast post and core.
- The use of a cast post and core without ferrule presents a lower occurrence of unfavorable failures. For teeth with a 0.5-mm-thick ferrule, both cast post and core and fiber post were associated with a similar percentage of unfavorable failures. For teeth with a 1-mm-thick ferrule, the use of the fiber post could be the appropriate clinical decision.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local oversight committee guidelines and policies of the Federal University of Santa Maria. The approval code for this study is 042272.

Conflict of Interest

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

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