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

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Academy of Operative Dentistry

Award of Excellence

Dr. Bruce W. Small

Dr. Bruce W. Small

It is my distinct pleasure and honor to be able to introduce this year's awardee of the Operative Academy's Award of Excellence to Dr. Bruce W. Small. I was supposed to give this presentation last year but a small pesky virus intervened so I am now finally able to award this belated honor to you at this 2022 AOD meeting. It is better late Bruce, than not at all.

Bruce graduated from the University of Medicine and Dentistry of New Jersey in 1973. Following graduation from dental school, he took a yearlong postgraduate residency after which he started his private practice in Lawrenceville, New Jersey, almost 50 years ago. He didn't know then that his dental career path would be changed forever by an event unforeseen by him at the time.

I first became acquainted with Bruce by a very circuitous route that started with a conversation with Fred Eichmiller, at an AAGFO meeting in Orlando, Florida, in the fall of 1993, in which he mention a person interested in gold by the name of Bruce Small. I thanked Fred for mentioning my name to Bruce as a potential speaker and promptly put the conversation in the "will never happen" bin. Much to my surprise and amazement, I received a call from Bruce asking if I would give a presentation on "gold" to the upcoming New Jersey Chapter of Cosmetic Dentistry meeting. I paused, gasped, and then the thought crossed my mind about how the Christians must have felt being tossed to the lions in the arenas across the Roman Empire. I was somewhat aghast but recovered my composure enough to say yes I would, and the title would be on "How to Make Gold Restorations Esthetic." I thought this title might placate the naysayers and perhaps peak the interest of others in the group.

I presented the lecture in the spring of 1994 in New Jersey, and it ended with a nice courteous applause from the meeting attendees. I figured that was that, until Bruce mentioned after the lecture that he would like to start a hands-on study club in his office. Now I was in a real pickle; do I take on this task or do I suggest someone else? I reluctantly told Bruce that I would help him start a study club, so a date was set for the first study club meeting later that year. When I arrived for the meeting in a suit and tie, the prospective member's attire was anywhere from beach dress to casual. I knew I had my work cut out for me to change the culture of this group. It was a motley crew, to say the least, but I now knew that I had long-term job security, if I so desired.

Over the next 5 years or so, the members started dressing like professionals, and their work improved accordingly. I thought this was an apropos time to suggest to Bruce that perhaps the club should look for another mentor, as it was a long distance to travel and the club was meeting eight times a year. Without hesitation, he answered, "I don't think so," and that was the beginning of a long friendship, both professionally and personally. I can regale you with many other stories about Bruce and I and the nights we held sway in his home on various topics, but I won't. Suffice it to say, out of these numerous evening soirees, with some wine or aperitifs and often a cigar, we developed a deep friendship, and I saw the beginning of a dental epiphany in Bruce. This was the beginning of his journey with alchemy and the transformation of composites and amalgams into beautiful long-lasting functional gold restorations for his patients.

In my experience, there are mainly three types of people one deals with throughout their lifetime. The first type makes things happen, the second type watches things happen, and the third type wonders what happened. After getting to know Bruce, maybe too well, I soon realized that he was the first type, which made things happen. He also showed a thirst and a quest to learn and improve his knowledge

and skills. He has taken more than 3000 hours of continuing education, not only to improve his skills and knowledge but also to improve his abilities to provide excellent care and treatment to his patients. He further has given over 400 continuing education courses and lectures to share this knowledge and skills with his fellow dentists. He has published more than 130 articles in various dental publications, many being on dental techniques, and has served as a reviewer on editorial boards. He presently teaches several hands-on dental study clubs, given many table clinics, given freely of his time to his alma mater, and has been lecturing for more than 25 years, nationally and internationally. He has served in the offices of many dental organizations and academies including our own Academy of Operative Dentistry.

As noted, Bruce has been a very active contributor to the profession of dentistry—perhaps one might say an overachiever. It has been an honor and privilege for me to have mentored and watched Bruce accept the challenges of his profession, master them, and pass them on to our younger colleagues. He has chosen the path in dentistry of the "harder right than the easier wrong." It is especially an honor for me to be asked by Bruce to make this presentation. Without further ado, it is my esteem pleasure to present today the Award of Excellence to a truly deserving person, Dr. Bruce W. Small, from the members of the Academy of Operative Dentistry for his outstanding contributions to excellence in dentistry and to this academy.

Warren K Johnson

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Clinical Technique/Case Report

Management of Localized Anterior Tooth Wear Using a Modified Sandwich Technique and the Dahl Concept: A Case Report

TW Lim • J Roffie

Clinical Relevance

A minimally invasive approach is proposed through the use of a modified sandwich technique combined with the Dahl concept to create interocclusal space and to eliminate the need for tooth structure removal of the affected dentition.

SUMMARY

This case report illustrates a minimally invasive segmental rehabilitation of localized anterior tooth wear using a modification of the sandwich technique, a combination of indirect palatal composite veneers and direct labial composite restorations, at an increased occlusal vertical dimension (the Dahl concept).

INTRODUCTION

The prevalence of tooth wear in young adults has dramatically increased in the last few decades.[1] Poor eating habits, parafunctional activities, and underlying medical conditions are risk factors for the development of tooth wear of nonbacteriological origin; this wear includes abrasion, erosion, attrition, and abfraction.[2] These etiological factors have resulted in loss of tooth structure, which can have biological, functional, and esthetic implications.

Traditionally, structurally compromised, worn-down dentition should receive multiple full-coverage crowns. However, this approach is expensive and technically challenging and requires much long-term maintenance care. Additionally, the vital tooth preparation for indirect restoration can involve significant removal of the compromised tooth structure and increases the risk of root canal treatment.[3] Therefore, the additive approach should be adopted in management of localized tooth wear. The lost tissues should be replaced using an adhesive material to preserve the remaining tooth structure.[4] Vailati and Belser[5] have introduced the anterior clinical erosive classification (ACE) based on their clinical observation of localized anterior tooth wear. This classification not only helps in assessing the severity of tooth structure loss but also provides a guide suggesting the type of restorations that the clinician

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should use to restore the affected teeth. The severity of tooth wear is graded in six levels (class I-class VI), based on the amount of dentin exposure on the palatal surface and the length of the remaining clinical crown height of the anterior teeth. Their recommendation was to manage the localized anterior tooth wear (Class IV and above) using sandwich-approach restorations, in which the worn-down surfaces are restored using a combination of indirect palatal composite veneers and labial porcelain veneers (Figure 1).

One of the main restorative challenges of localized tooth wear is lack of interocclusal space. Therefore, the Dahl concept has been recommended to create space for definitive restorations.[6] It is defined as "axial tooth movement observed when a localized appliance or restoration was placed in supra-occlusion and full arch occlusal re-establishment contact occurred over a period of time."[7] Dahl and others[6] were the first to introduce this concept for the management of localized worn dentition. They used a removable partial cobalt-chromium bite plane prosthesis to create interocclusal space in a young patient with localized severe tooth wear. Sufficient space was created after a period of 8 months to restore the worn dentition. Since then, there have been more and more clinical studies reporting the application of the Dahl concept and the use of various Dahl appliances to create interocclusal space for tooth wear cases.[7] Occlusal re-establishment is defined as a physiological compensatory process that occurs gradually in most patients to ensure that occlusal contacts are obtained and the efficacy of the masticatory system is maintained.[7] The physiological process involves intrusion of the teeth in contact with the prosthesis, which is cemented at an increased occlusal vertical dimension (OVD), eruption of the unopposed teeth, and an element of mandibular repositioning.[8,9] Most of the clinical studies have reported high success rates, ranging from 94% to 100%, of occlusal re-establishment in localized tooth-wear cases.[8-10]

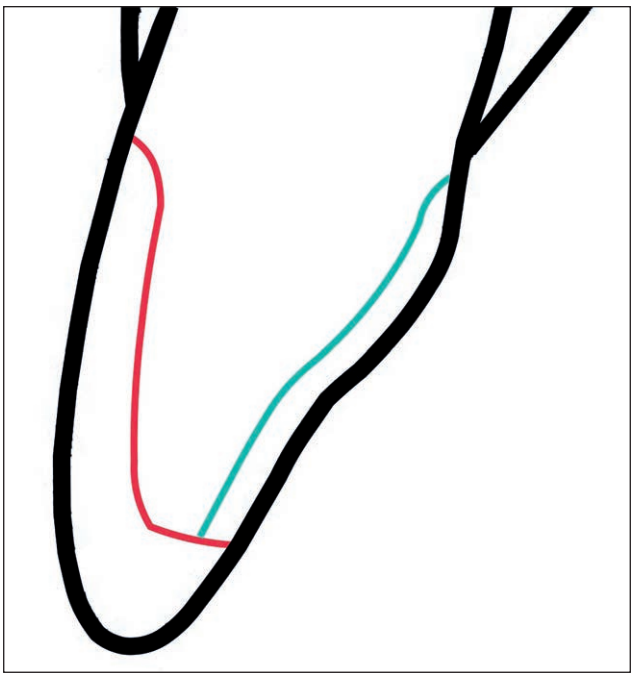

Figure 1. *The sandwich approach using a combination of indirect palatal composite veneers and labial porcelain veneers.*

The present clinical report describes occlusal therapy of localized anterior tooth wear using the Dahl concept. The Dahl restorations, a combination of indirect hybrid composite veneers on palatal surfaces and direct resin composites on the labial side (modified sandwich technique) were delivered to the patient at an increased (OVD).

METHODS AND MATERIALS

Case History

A 33-year-old female presented with a chief complaint of anxiety over her appearance due to tooth wear. On further questioning, she revealed that she had nocturnal bruxism and had been diagnosed with hyperemesis gravidarum during her two pregnancies. She denied any excessive consumption of acidic food or beverages. Her medical, dental, social, and family history was unremarkable. She has a high smile line with upper lip canted to the right side while smiling (Figure 2a). The intraoral examination revealed localized moderate-to-severe tooth wear on her maxillary and mandibular anterior teeth, with predominant attrition and erosion (Figure 2). Identical wear facets between maxillary and mandibular incisors were seen during left lateral excursion, suggesting a wear pattern related to parafunctional habits (Figure 3).

Restorative Treatment

During the treatment planning stage, pulp tests, including cold and electrical pulp testing, showed positive responses for all maxillary anterior teeth. An interocclusal record was taken in centric relation using extra-hard wax (Moyco Beauty Wax, Dentsply Sirona, Charlotte, NC, USA) and marked using temporary cement material (Temp-Bond, Kerr, Brea, CA, USA) (Figure 4a). A Lucia jig was used as a muscle deprogrammer and anterior stop (Figure 4b). The study casts were mounted on an arcon semi-adjustable articulator (Denar Mark II system, Whip Mix Corp., Louisville, KY, USA) using a facebow transfer (Denar Slidematic Facebow, Whip Mix Corp.). Diagnostic wax-up of the anterior teeth at an increased OVD of 2

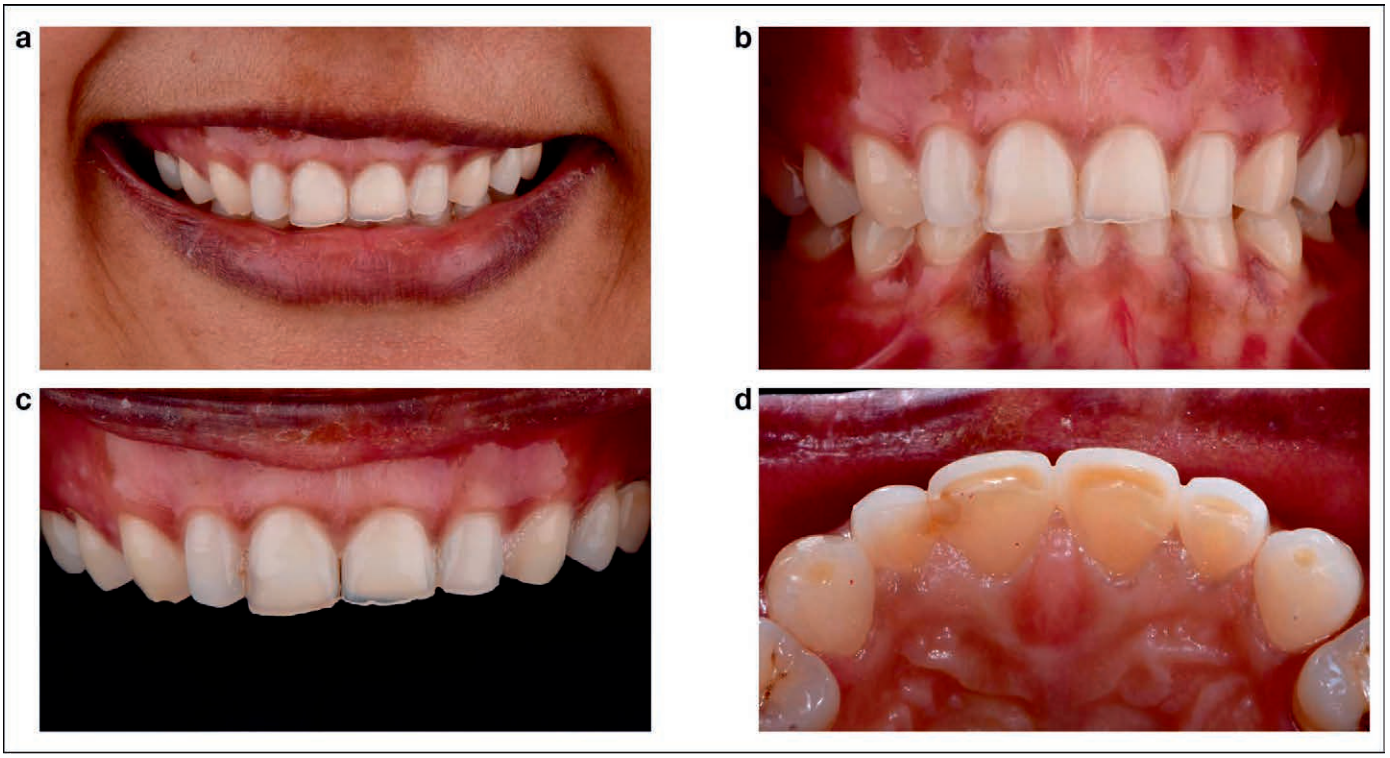

Figure 2. *Preoperative photographs: a) Smiling view; b) Erosion and attrition found in maxillary incisors; c) Accentuated translucency of the maxillary incisors; d) Maxillary occlusal view showing erosion of maxillary anterior teeth.*

mm was performed (Figure 5). The patient was satisfied after seeing the trial mock-up of the treatment outcome (Figure 6). The increased OVD was established by evaluation of esthetics, occlusion, and phonetics during the mock-up visit.

Restorative treatment was conducted using a modified sandwich technique with minimal tooth preparation limited to removal of unsupported enamel. Sectional metal strips were placed between the teeth to separate them prior to the impression (Figure 7a). A definitive impression of the maxillary arch was taken using polyvinylsiloxane material (Aquasil, Dentsply)

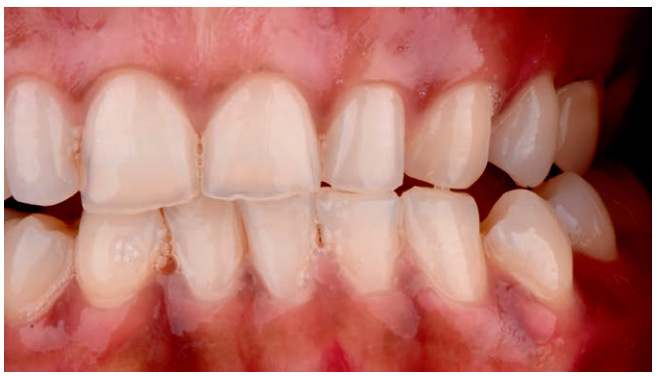

Figure 3. *The identical wear facets of the maxillary and mandibular incisors during left lateral excursion.*

(Figure 7b). The veneers were fabricated using micro-ceramic indirect resin composite material (Ceramage, Shofu, Kyoto, Japan) because of its high inorganic content, which is indicated for use in areas subjected to greater masticatory loads (Figure 8). This indirect restorative material is a micro-ceramic polymer system with a composition of 73% zirconium silicate filler (PFS-progressive fine structured filler) supported by an inorganic polymer matrix containing urethane dimethacrylate and urethane diacrylate.

Shade selection was performed prior to any treatment (Figure 9a). The maxillary teeth were then isolated using a rubber dam (Figure 9b). The palatal veneers were sandblasted with 50 μm aluminum oxide particles for 10 seconds, at a distance of 10mm using two pressure bars, followed by a steam clean to remove debris. The fitting surfaces of the veneers were then treated using a silane coupling agent (Ultradent, South Jordan, UT, USA) and application of an adhesive system (Scotchbond Universal Adhesive, 3M Oral Care, St Paul, MN, USA) without curing. The palatal enamel and dentin were etched for 15 seconds using 37% phosphoric acid, followed by application of the adhesive system (Scotchbond Universal Adhesive, 3M Oral Care), which was applied for 20 seconds without curing. Subsequently, the palatal veneers were cemented using preheated resin composites (ENA

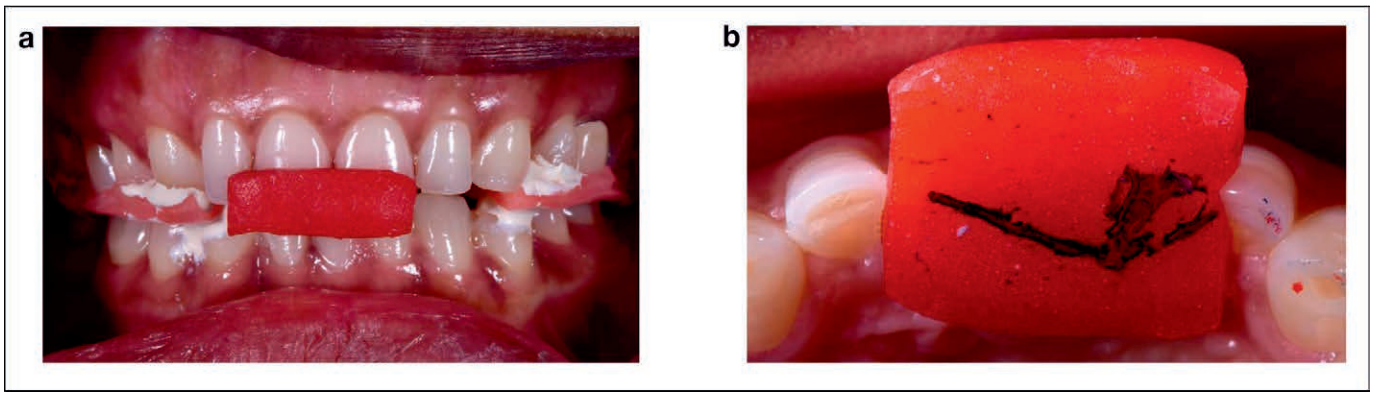

Figure 4. *The interocclusal record: a) Interocclusal record taken at centric relation; b) Lucia jig.*

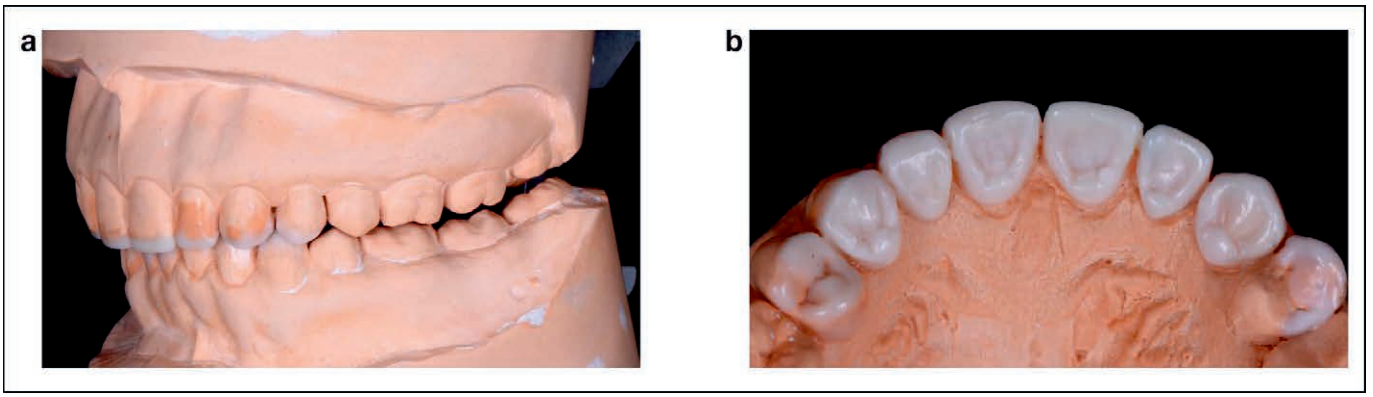

Figure 5. *The diagnostic wax-up: a) The diagnostic wax-up at an increased occlusal vertical dimension; b) Diagnostic wax-up of maxillary palatal surface.*

HRi, Micerium, SYNCA, Le Gardeur, QC, Canada) and light cured for 20 seconds. The resin composites for cementation were warmed for 15 minutes at 54°C using a composite heating conditioner (ENA Heat, Micerium, SYNCA). As a result, the increase in OVD could be obtained in a predictable way following the planned occlusion with the prescribed anterior guidance. In order to ensure preservation of remaining tooth structure, a modified sandwich technique was adopted. The labial surfaces of the indirect palatal veneers were then cut back and acted as palatal shells for labial resin composite buildup (Filtek Z350 XT, 3M Oral Care) (Figure 10). This is a nanofilled resin composite containing bisphenol-A-diglycidylether methacrylate, urethane dimethacrylates, triethylene glycoldimethacrylate, ethoxylated bisphenol-A-glycol dimethacrylate resins, silica and zirconia fillers, and aggregated zirconia/ silica cluster fillers. After the restorations were completed, white stone bur polishing discs (Sof-Lex, 3M Oral Care) and polishing pastes (ENA Shiny, SYNCA) were used for finishing and polishing (Figure 11).

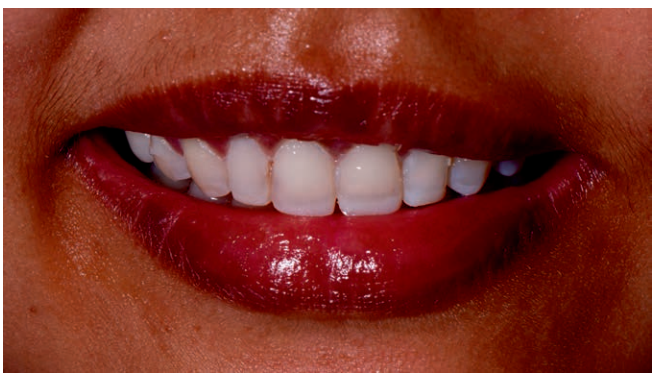

Figure 6. *Trial mock-up.*

All of the maxillary anterior restorations were placed at an increased OVD using the Dahl concept. Postoperative instructions were given to the patient, the possibility of discomfort and difficulty in chewing were discussed, and a temporary soft diet regime was advised (Figure 12). The patient was scheduled for a 3-month follow-up appointment. Occlusal analysis exhibited complete occlusal re-establishment, with Shimstock foil (Bausch Arti-Fol, Bausch, Nashua, NH, USA) holding between all the posterior teeth (Figure 13).

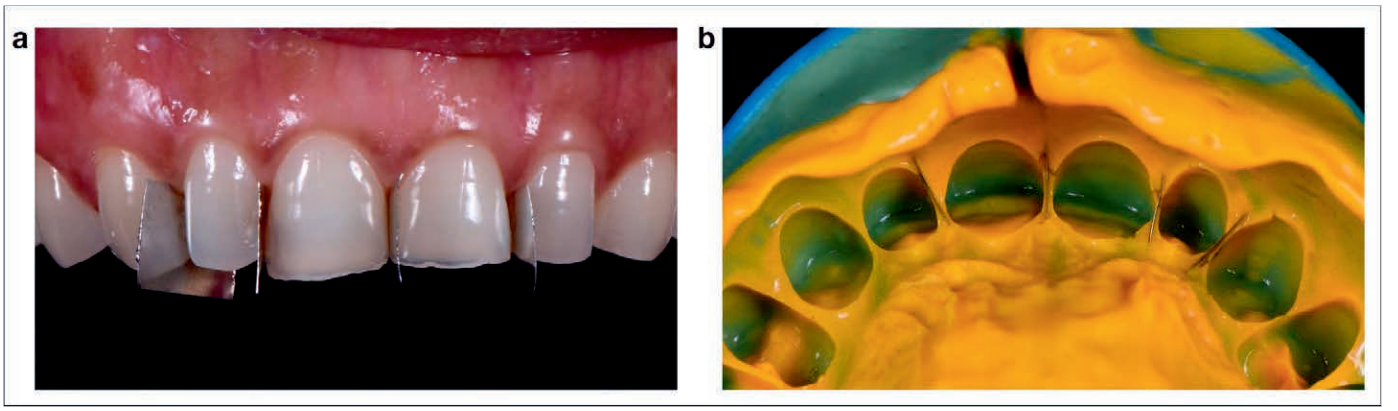

Figure 7. *Definitive impression: a) Sectional metal strips were placed in between the teeth; b) The definitive impression was taken using dual-impression technique.*

List of Materials Used

- Extra-hard wax (Moyco Beauty Wax, Dentsply Sirona, Charlotte, NC, USA)
- Arcon semi-adjustable articulator (Denar Mark II system, Whip Mix Corp., Louisville, KY, USA)
- Facebow (Denar Slidematic Facebow, Whip Mix Corp.)
- Polyvinylsiloxane (Aquasil, Dentsply)
- Ceramage indirect resin composite (Shofu, Kyoto, Japan)
- Silane coupling agent (Ultradent, South Jordan, UT, USA)
- Scotchbond Universal Adhesive (3M Oral Care, St Paul, MN, USA)
- ENA HRi, Micerium (SYNCA, Le Gardeur, QC, Canada)
- Filtek Z350 XT (3M Oral Care)
- Sof-Lex discs (3M Oral Care)
- ENA Shiny (SYNCA)
- Shimstock foil, Bausch Arti-Fol (Bausch, Nashua, NH, USA)

Figure 8. *The indirect palatal hybrid composite veneers were fabricated.*

DISCUSSION

Potential Problems

Preservation of tooth structure should be the paramount aim in tooth wear management, especially for a young patient. Therefore, the traditional prosthodontic approach should be avoided to prevent any tooth preparation on the worn-down dentition. The recommended sandwich technique for restorations, using a combination of indirect resin composites on the palatal surfaces and porcelain veneers on the labial surfaces, have shown great potential for adoption as an additive approach for tooth wear management.[11-13] However, the modified sandwich technique described in the present clinical report illustrates a more conservative approach, without any tooth preparation, yet achieving an excellent esthetic and functional outcome. All resin composites have technical requirements that include moisture control, precise adhesive procedures, and superior dexterity. Additionally, a high level of maintenance care can be expected for resin composites because the restorations will undergo wear and mechanical complications, including fracture and discoloration over time. Therefore, the patient should be scheduled for regular follow-up visits (Figure 14).

There have been a few clinical studies reporting that biological complications rarely occur after application of the Dahl concept. Poyser and others[7] have reported a small number of long-term adverse effects including temporomandibular disorder, pulpal symptoms, periodontal symptoms, and root resorption as consequences of Dahl restoration. A potential drawback of this technique might be partial occlusal re-establishment. However, most of the patients reported that they function perfectly well with the reduced number of occlusal contacts. Gulamali and others[14] found that 5 out of 26 (19%) localized anterior tooth wear

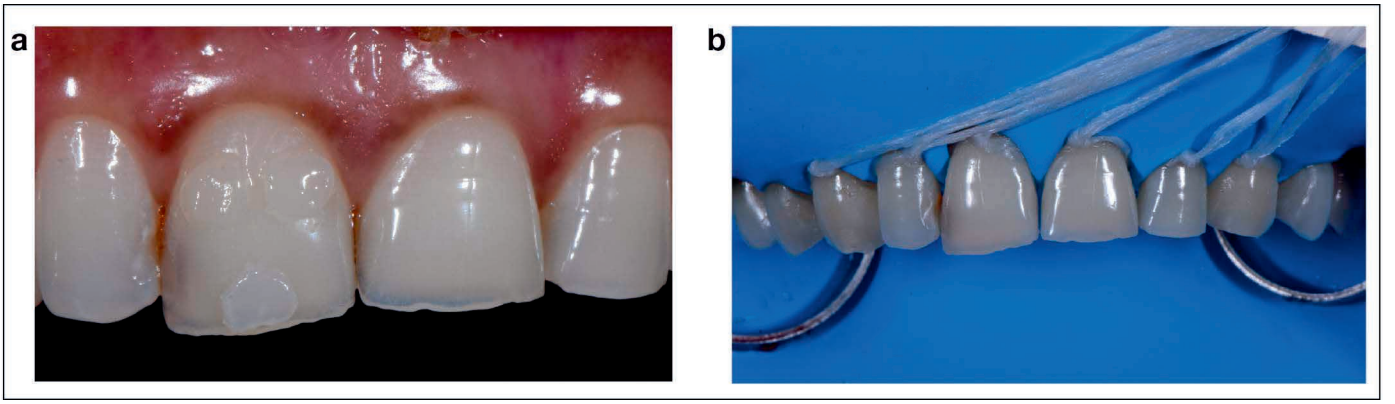

Figure 9. *Before restorations: a) Shade selection;. b) Rubber dam isolation.*

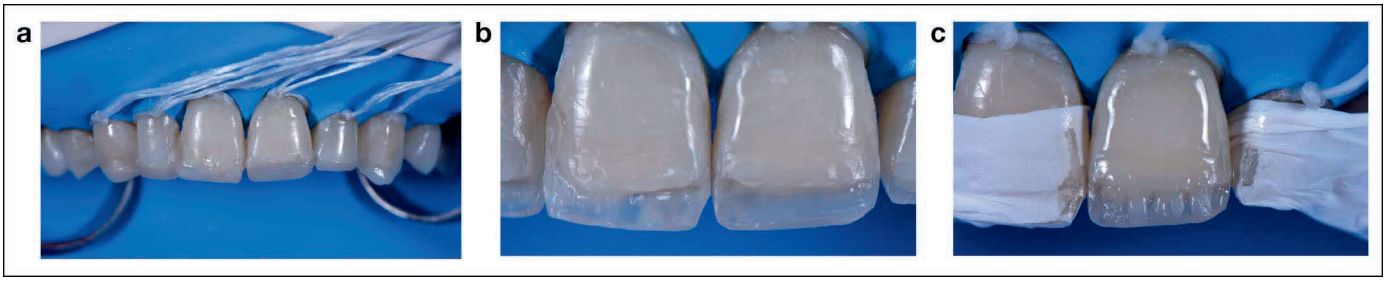

Figure 10. *Indirect palatal veneer cementation: a) Post-cementation of the palatal veneers. b) Palatal veneers cut back and used as palatal shells for buildup of labial resin composites; c) Layering resin composite technique applied.*

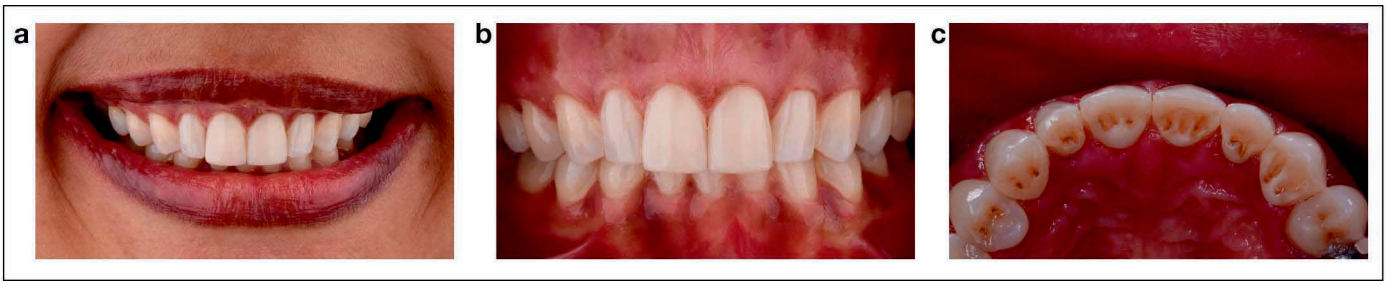

Figure 11. *Postoperative photographs: a) Smiling view; b) Labial view; c) Occlusal view.*

patients exhibited partial occlusal re-establishment of their posterior teeth at 10-year follow-up and that all of them were asymptomatic. Overlay preparation or direct resin composites applied to the posterior teeth are suggested in case no occlusal re-establishment occurs after placement of the Dahl restorations.[15] In the present case, full occlusal re-establishment was achieved within 3 months, consistent with previous clinical studies that revealed 94% to 100% success rates of occlusal contact re-establishment in localized tooth wear cases within four to nine months. [8-10]

Summary of Advantages and Disadvantages

In comparison with a traditional full-mouth rehabilitation, a simplified ultraconservative approach is suggested, using an occlusal therapy that combines the Dahl technique and a modified sandwich technique to manage localized tooth wear in a safe, simple, cost effective, and minimally invasive way. The main advantage of the modified sandwich technique in the present case report is that there was no tooth preparation on the compromised tooth structure. Direct resin composites were used in place of the porcelain veneers previously suggested by Vailati and Belser,[5] which still require tooth preparation on the labial surfaces. The long-term survival rates of direct resin composites in tooth wear management have shown no statistically significant difference from full-coverage indirect restorations.[16] In addition, there have been several clinical studies that reported the successful placement of resin composite restorations to treat localized anterior tooth wear by using the Dahl concept.[8,9,14]

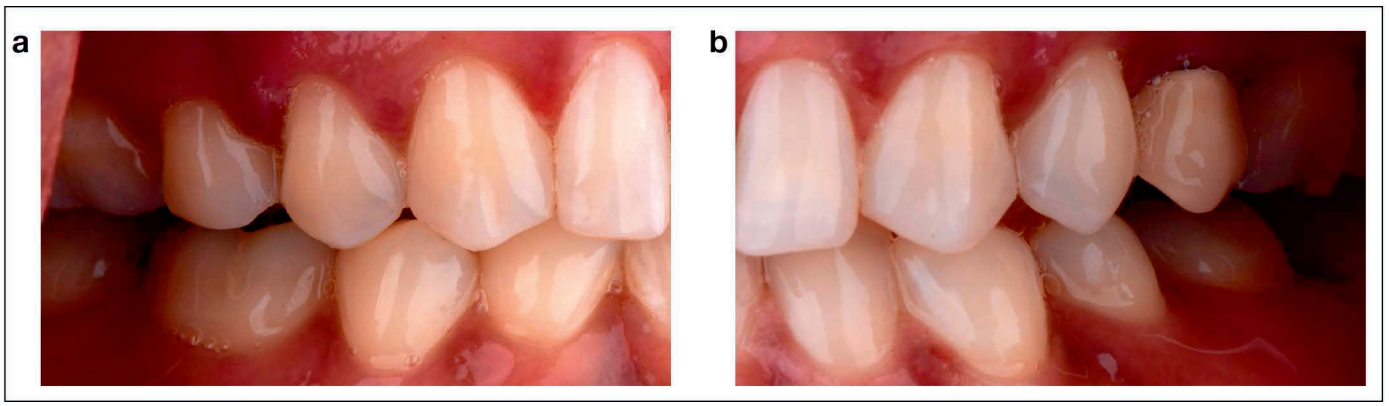

Figure 12. *Immediate postoperative intraoral photographs: a) Right buccal view with no posterior occlusal contacts; b) Left buccal view with no posterior occlusal contacts.*

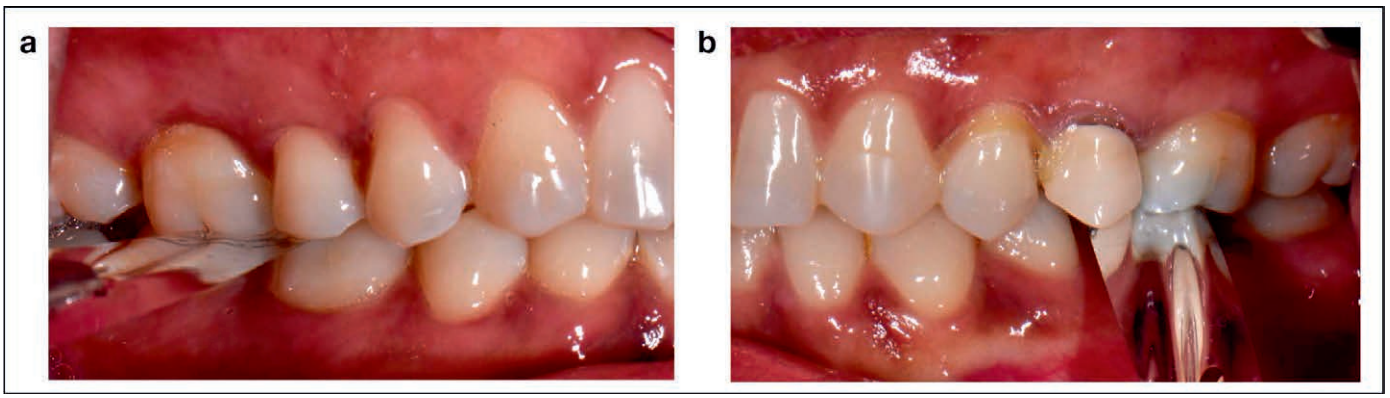

Figure 13. *Intraoral photographs taken at 3-month follow-up appointment: a) Right buccal view with Shimstock foil hold; b) Left buccal view with Shimstock foil hold.*

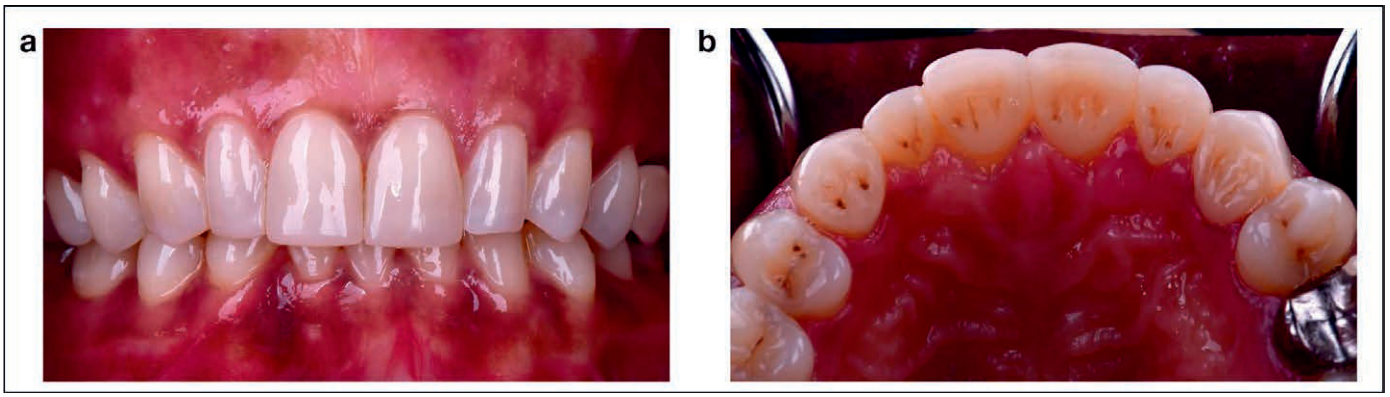

Figure 14. *Intraoral photographs taken at 18-month follow-up appointment: a) Labial view; b) Occlusal view with evidence of wear on indirect resin composites.*

Indirect hybrid composites provide high fracture resistance, natural color replication, outstanding polishability, as well as good plaque resistance.[17] The indirect technique itself offers many benefits compared to direct restorations in terms of better mechanical performance and a significant reduction in polymerization shrinkage.[18] Additional clinical benefits include precise marginal integrity, ideal static and dynamic occlusal contacts, excellent anatomic morphology, and optimal esthetics.[19] The longevity of resin composites is probably their main disadvantage. However, Hemmings and others[8] reported that severe localized tooth wear managed using hybrid resin composites showed low failure rates after a mean

follow-up of 30 months. Bruxism is a risk factor strongly associated with mechanical complications in oral rehabilitation.[20] Resin composite restorations in bruxers demonstrated three to four times more substance loss than ceramics.[20,21] However, Willems and others[22] demonstrated that modern composite resin restorations have similar wear rates to human enamel. Therefore, the use of resin composite restorations to treat localized anterior tooth wear at an increased OVD is a viable treatment option that requires some degree of maintenance.

Conflict of Interest

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

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Clinical Research

36-Month Randomized Clinical Trial Evaluation of Preheated and Room Temperature Resin Composite

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Clinical Relevance

The results of this study confirmed that clinicians can consider using preheated composites in dental practices. The study also found evidence of better clinical performance regarding marginal staining when applying preheated composites. Considering that postoperative sensitivity was reduced over time, its use in routine care can be considered a good practice.

SUMMARY

Objective: This study evaluated the effect of preheating resin composites (RCs) on the clinical performance of class I restorations during a 36-month period using a split-mouth, double-blinded randomized design.

Methods and Materials: A total of 35 patients were selected. Every patient received one pair of class I nanofilled resin composite (RC, Filtek Z350 XT) posterior restorations (n=70). One side of the mouth received preheated composites; on the other side, the composite was placed in a nonheated state following the manufacturer's instructions. These restorations were evaluated at 1-week (baseline), 12-months, 24-months, and 36-months using the FDI World Dental Federation criteria. The statistical analyses were also performed using the Wilcoxon and Friedman tests with the level of significance set at 0.05.

Results: After 36 months, 33 patients attended the recall visits, and 66 restorations were evaluated. The Friedman and Wilcoxon signed-rank tests revealed insignificant differences between both groups (p>0.05) for all FDI parameters. However, a significant difference was detected for staining as a criterion at 36 months (p=0.01). Moreover, a significant difference in the staining was detected when the baseline and 36 months were compared

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in the nonheated RC group (p=0.001). For esthetic, functional, and biological properties, the nonheated composite exhibited 93.9%, 100%, and 100% of the clinically accepted scores, respectively, and the preheated group presented 100% for all properties. Four restorations had postoperative sensitivity at baseline for nonheated (11.4%) and five for preheated (14.2%), but the postoperative sensitivity scores were considered highly acceptable at 12-, 24-, and 36-months.

Conclusions: After 36 months, preheated nanofilled RCs showed an acceptable clinical performance similar to that of the nonheated ones in class I restorations, but with better resistance to marginal staining.

INTRODUCTION

Composite materials, with their excellent mechanical and esthetic properties, have been successfully utilized for many years as dental restorative materials.[1] Preheating resin composites (RCs) has a crucial effect on the polymerization of multifunctional monomers, which are the prime components of methacrylate-based restorative materials.[2] Furthermore, the mobility of free radicals and monomers is enhanced by increasing the polymerization temperature, and a higher overall conversion thus occurs, which, in turn, leads to improved mechanical, physical, and surface properties of preheated RCs.[3]

Composition and microstructure are accountable for the mechanical properties of RCs.[4] Adequate clinical performances together with the enhanced mechanical properties of RCs have also made them more suitable for posterior restorations.[5] Hence, practitioners might consider preheating RCs for increasing handling characteristics, with the expectation that mechanical properties will be improved.[6]

Laboratory research suggests that preheating RCs before placement can have significant clinical advantages,[7] such as improved rheological properties and reduced film thickness,[8] enhanced adaptation and reduced microleakage,[9] greater monomer conversion during polymerization,[7,10,11] reduced curing time,[7] increased hardness,[9,12] and enough flowability to lute porcelain laminate veneers.[13] Despite these improved properties, the technique of preheating RCs is not widely accepted. One possible reason for the reluctance of dental clinicians to use preheated RCs is the lack of sufficient clinical evidence when using this technique. Thus, the rationale behind this study was to prove whether or not a preheating procedure provides more advantages regarding clinical situations.[14]

The preliminary assessment of any restorative material is conducted using laboratory investigations, but clinical studies are more important in evaluating its performance.[15,16] Thus, several variables (mastication forces, temperature fluctuations, humidity variations, and salivary enzymes) could influence the overall performance of a restorative material.[17] The majority of studies performed on preheated RCs are laboratory ones demonstrating improved properties when dental RCs are preheated.[18-21] These improved rheological properties, increased hardness, improved adaptation, and reduced microleakage may or may not reduce postoperative sensitivity. Hitherto, a review of the literature has revealed that there is only one reported randomized controlled clinical trial of postoperative sensitivity to evaluate the clinical performance of preheated RCs.[14]

Thus, the present study was designed to provide further evidence in this particular research perspective, and this study aimed to evaluate the effect of preheating RCs on the clinical performance of class I restorations in a 36-month period. The formulated null hypothesis was that there is no significant difference in the clinical performance of preheated RCs in comparison with nonheated RCs in a 36-months period in class I restorations.

The research question was as follows: Do preheated RCs in class I restorations present better clinical performances than nonheated RCs according to the FDI World Dental Federation criteria?

METHODS AND MATERIALS

Restorative Materials and Curing Device

A nanofilled RC restorative system (Filtek Z350 XT) with Single Bond Universal adhesive (3M ESPE, St Paul, MN, USA) was utilized in the present study and applied according to the manufacturer's instructions. Table 1 presents its specifications. A light curing unit (LED Bluephase C5, Ivoclar, Vivadent, Amherst, NY, USA) with an output density of 655 mW/cm^2 was applied. The intensity of the light curing unit was measured periodically using Demetron LED light meters (Demetron Research Corp, Danbury, CT, USA).

Study Design

The description of the experimental design followed the Consolidated Standards of Reporting Trials statement.[22] The present study was a double-blind (patients and examiner) randomized clinical trial anticipating the split-mouth design.

Table 1: *Restorative Materials and Application Procedures According to the Manufacturer's Instructions*

Restorative System	Manufacturer	Composition	Lot Number	Application Procedure
Filtek Z350 XT	3M ESPE, St Paul, MN, USA	Treated silanized ceramics; silane- treated silica; urethane dimethacrylate; bisphenol A polyethylene glycol diether dimethacrylate; bisphenol A-glycidyl methacrylate; ceramics of zirconia; polyethylene glycol; dimethacrylate; triethylene glycol; and dimethacrylate	N625490	Resin composite applied incrementally up to two increments and each increment was photopolymerized individually
Single Bond universal Adhesive	3M ESPE, St Paul, MN, USA	Methacryloyloxydecyl dihydrogen phosphate; phosphate monomer; dimethacrylate resins; hydroxyethyl methacrylate; methacrylate-modified polyalkenoic acid copolymer; filler; ethanol; water; initiators; silane	517577	Adhesive was applied using etch-and-rinse strategy. Total etching with 36% phosphoric acid (enamel 30 s, dentin 15 s) followed by rinsing with water for 20 s and drying with air free of moisture and oil, without drying out for 5 s. Adhesive application to tooth surface by scrubbing action (20 s), drying of the adhesive (5 s), and light curing (10 s)

Patient Selection

Thirty-five adult patients seeking dental treatment in the Operative Department Clinic at the Faculty of Dentistry, University of Mansoura were enrolled in the present study with a total of 70 class I restorations. No advertisement was made for participant recruitment, forming a sample of convenience. Additionally, each patient signed a consent form before participating in the present study. The study was conducted from October 2017 to June 2020 as a part of a doctoral dissertation, and the trial was registered by ClinicalTrials.gov. No protocol deviations emerged during the trial.

Sample Size Calculation

The sample size was calculated on the basis of the clinical success rate (100% retention rate in 36-months) of posterior class I restorations restored with nanofilled composites observed in a previous study.[23] According to several parameters, including a significance level of 5%, the power of the test was calculated to be 80% and the equivalent limit to be 15%. Based on these data, a sample size of 30 subjects was appropriate, and allowing for a 20% drop-out, a sample size with a total of 35 subjects was set.

Eligibility Criteria

The inclusion criteria included the presence of primary caries involving occlusal surfaces only and International Caries Detection and Assessment System (ICDAS) 2 or 3 with cavities no more than one-third of the intercuspal distance. No third molars were selected. Patients needed to have good oral hygiene; the selected tooth needed to give a positive response to testing with an electric pulp tester and with normal and full occlusion, having opposing natural teeth with no restorations. High caries risk patients with extremely poor oral hygiene and patients involved in orthodontic treatment or periodontal surgery, and periodontally involved teeth (chronic periodontitis) and abutments were excluded. Moreover, patients with heavy bruxism habits, clenching, and evidence of wear facets on teeth were excluded. Tables 2 and 3 exhibit the data regarding the characteristics of patients and restored cavities.

Random Sequence Generation and Allocation Concealment

Each patient received one pair of class I posterior restorations, a preheated and a nonheated RC

Table 2: *Number of Lesions According to Sex and Age of Patients*

Characteristics of Patients	Number of Lesions
Sex	
Female	19
Male	14
Age, y	
20-25	20
25-30	8
30-35	5

Table 3: *Characteristics of Restored Cavities*

Characteristics of Restored Tooth	Number of Lesions	
	Nonheated	Preheated
Teeth distribution		
Premolars	12	13
Molars	23	22
Dental arch distribution		
Upper	15	12
Lower	20	23
Pulp protection		
Yes	0	0
No	35	35
Presence of antagonist		
Yes	35	35
No	0	0
Width		
Small	10	8
Medium	16	19
Large	9	8
Depth		
Shallow	2	3
Medium	33	32
Deep	0	0
Reason for restoration		
Fracture	2	1
Caries	26	29
Caries and fracture	2	0
Esthetics	5	5

restoration, each in different sides of the mouth (split-mouth design). Hence, they were placed randomly in the two cavities of each pair (35 pairs) and determined by using online software (www.sealedenvelope.com). A blocked list was generated, and a randomization code was performed according to two treatment possibilities (preheated and nonheated). The cavities within the pair were also chosen to match each other concerning size and localization. However, the patients remained blind to the allocation at all times. A staff member not involved in the clinical trial prepared the envelopes.

Clinical Procedures

One experienced operator prepared, restored, and finished 70 class I nanofilled Filtek Z350 XT RC restorations either preheated or nonheated, and the adhesive cavity design was utilized. Initially, the patients were given local anesthesia with 1.8 mL of 2% lidocaine hydrochloride and phenylephrine 1:2500 (SS White 100, SS White, Petropolis, Brazil) before the restorative procedures to reduce discomfort. Fissure carbide burs and round diamond stones (Komet, Brasseler GmbH Co. KG, Lemgo, Germany) were utilized to prepare cavities at a high speed with copious water cooling followed by excavation of remaining caries using tungsten carbide burs (Komet, Brasseler GmbH Co. KG) at a low speed and sharp excavators. After the shade selection, a rubber dam with high suctioning was also utilized to isolate the operative field. The enamel was selectively etched with 36% phosphoric acid gel (Scotchbond Etchant, 3M ESPE, St Paul, MN, USA) for 30 seconds while the dentin was etched for 15 seconds. The preparation was then thoroughly rinsed for 20 seconds and gently air dried. A disposable brush was used to apply the adhesive by a scrubbing action for 20 seconds followed by gently air drying for 3-5 seconds and then light cured using a light curing unit for 10 seconds.

A device called Therma-flo RC warming kit (Vista, WI, USA) was applied for the heating of RCs according to the manufacturer's instructions. The warming device was operated for 30 minutes until it reached 68°C, and then the syringe tube was placed inside a heating chamber for 5 minutes to reach the temperature of the warming device. The syringe was then removed from the device, and the RCs were applied immediately using gold-plated instruments. The teeth were restored incrementally up to two increments in the form of oblique layers, with each increment being up to 2 mm. Consequently, each increment was light cured for 25 seconds, and the restorations were finished and contoured at the same visit using low-speed fine-grit diamond finishing stones (Komet, Brasseler GmbH Co. KG) and copious

amounts of water as a coolant. Furthermore, the occlusal morphology was established using articulating paper (Bausch, Nashua, NH, USA). The polishing procedures were performed immediately using Soflex discs (3M ESPE) in a recommended order (coarse, medium, fine, and superfine) with water coolant to obtain a smooth surface.

Calibration Procedures for Clinical Evaluation

In October 2017, two examiners who did not participate in the placement of the restorations were trained for the evaluation process using an online calibration tool (www.e-calib.info). An inter-examiner and intra-examiner agreement before the beginning of the evaluation of at least 90% was requested.[24]

Blinding

The examiners not involved in the placement of restorations and the participants were both blinded to the intervention; hence, this study was categorized as a double-blind study.

Clinical Evaluation

All the restorations were evaluated clinically two weeks after finishing and polishing procedures (baseline) and after 12-, 24-, and 36-months using the World Dental Federation FDI criteria.[25] The clinical intraoral photographs were taken at all recall periods, and the standardized case report for each patient was applied to record the FDI parameters during the evaluation procedures. After each observation, the case report forms were sent to the research staff to ensure that all the evaluators were blinded to the group assignment along with the follow-up recall visits.

Only the clinically relevant measures of the performances for RCs in class I restorations were evaluated. The primary clinical endpoints were marginal adaptation, surface and marginal staining, restoration retention, and fractures, but the following secondary outcomes were also evaluated: surface luster, color match, postoperative sensitivity, tooth integrity, patient's view, and recurrence of caries. The parameters that required clinical visibility were evaluated using a magnifying dental loupe with a magnification of 4.3× and a working distance of 40 cm (EyeMag Pro F, Carl Ziess Meditec Ag, Germany) with a powerful illumination intensity from a light source attached to the loupe (EyeMag Light II, Carl Ziess Meditec Ag). The primary and secondary clinical endpoints were also ranked using the following scores according to the FDI criteria: (clinically very good, clinically good, clinically satisfactory, clinically unsatisfactory, and clinically poor).

Statistical Analysis

The statistical package program (IBM-SPSS version 26.0, IBM, Armonk, NY, USA) was utilized for the tabulation, coding, and analysis of the data. Descriptive statistics were also applied to describe the evaluated data distributions. Additionally, the Friedman test was used for intragroup comparisons between the baseline and other periods, whereas the Wilcoxon signed-rank test was utilized to compare both groups in each period. The comparisons were also performed for all the criteria evaluated with a significance level of 5%, and the Cohen kappa statistic was used to measure the agreement between the examiners.

RESULTS

All the restorative procedures were implemented exactly as planned, and no further modifications were performed. Recall rates were 100% for baseline, 12, and 24 months. At 36-months, the recall rate was 94.3%; two patients did not attend due to health problems. Table 4 shows the clinical scores according to the FDI evaluation criteria. Regarding the agreement between the examiners, the overall Cohen κ statistics revealed a satisfactory agreement between all the examiners at baseline (0.94), 12-months (0.95), 24-months (0.96), and 36-months (0.93). Staining (marginal and surface) was detected in three restorations in the nonheated group (9.6%) and marked staining was observed in two restorations in the same group (6.5%). Therefore, staining showed a significant difference between the nonheated RC and preheated RC groups (p=0.01). Additionally, a significant difference in staining was detected when the 1-week (baseline) and 36-month time point were compared in the nonheated RC group (p=0.001). Four restorations had postoperative sensitivity at baseline for the nonheated group (11.4%) and five for the preheated group (14.2%), but the postoperative sensitivity scores were considered highly acceptable at the 12-, 24-, and 36-month evaluation periods. Generally, the Friedman and Wilcoxon signed-rank tests revealed insignificant differences between the preheated and nonheated RC groups (p>0.05) for all the FDI parameters except that a significant difference was detected for the staining criterion at 36-months. For esthetic, functional, and biological properties, the nonheated composite revealed 93.9%, 100%, and 100% of the clinically accepted scores, and the preheated group presented 100% clinically accepted scores for all the properties.

DISCUSSION

Studying the preheating effect on the mechanical properties of nanofilled RCs in class I restorations

Table 4: *Summary of FDI Clinical Criteria Findings of Nonheated and Preheated Filtek Z350 XT RC over a 36-Month Follow-up Period*

Esthetic Properties	Nonheated Z350 XT					Preheated Z350XT			
	Score	Baseline	12-M	24-M	36-M	Baseline	12-M	24-M	36-M
Surface luster	1	35	30	25	22	35	29	24	21
	2	0	5	10	11	0	6	11	12
Staining									
Surface	1	35	34	33	24	35	35	35	32
	2	0	1	2	4	0	0	0	1
	3	0	0	0	3	0	0	0	0
	4	0	0	0	2	0	0	0	0
Marginal	1	35	34	33	23	35	35	35	32
	2	0	1	2	5	0	0	0	1
	3	0	0	0	3	0	0	0	0
	4	0	0	0	2	0	0	0	0
Color match and translucency	1	30	30	29	26	30	30	29	26
	2	5	5	6	7	5	5	6	7
Functional properties									
Marginal adaptation	1	35	35	35	32	35	35	35	32
	2	0	0	0	1	0	0	0	1
Biological properties									
Postoperative (hypersensitivity) and tooth vitality	1	31	35	35	32	30	35	35	32
	2	2	0	0	1	3	0	0	1
	3	2	0	0	0	2	0	0	0

Abbreviations: 1, clinically very good; 2, clinically good; 3, clinically satisfactory; 4, clinically unsatisfactory.

provides valuable information to clinicians to promote using RCs in a more flowable form. The class I restorations were selected to easily standardize the cavity dimensions and C-factors. Hence, in a clinical situation, the viscosity of RCs is reduced upon preheating, offering a more flowable state that can be injected into cavity preparations rather than using conventional hand instruments for RC manipulation.[26] Thus, a warm RC technique guarantees better handling properties, gaining the advantages of the outstanding mechanical, wear, and surface properties of nanofilled RCs.[27]

The authors[28] reported a 50% drop in temperature within two minutes in the RC samples upon the removal from the heating device. Hence, the authors suggested that clinicians must work very quickly to ensure the least temperature drop possible when using a heating device for the best clinical performance.[28] Stabilizing the temperature until the light curing process is thus of ultimate importance. Consequently, the RC temperature was strictly standardized in the present study, as the insertion time to the mold or cavity preparation was 40 seconds and the curing time was 25 seconds but the overall 65 seconds may have reduced the RC temperature.

Several *in vitro* studies have demonstrated the possibility of preheating to enhance the physical and mechanical properties of RCs.[29] However, the data regarding the clinical performance of preheated RCs compared with nonheated RCs are scarce; thus, the present study is of interest. Multiple factors affect the oral environment as temperature changes, such as bacterial flora, pH alterations, and occlusal stresses. These factors differ from patient to patient, which makes reproducing oral physiology profoundly difficult. Thus, the present study anticipated the split-mouth design. Although *in vitro* studies may give useful information regarding the physical and mechanical properties of restorative materials, they still cannot estimate the clinical handling properties or the clinical

performance of restorative materials. Subsequently, the clinical oral environment is considered the most useful way to assess restorative techniques and restorative dental materials.[30] The clinical evaluation process requires reliable, relevant, and objective criteria for assessing the clinical performance of restorative materials precisely.[25] Hence, the FDI clinical evaluation criteria were chosen.[25]

A significant difference was found between nonheated and preheated RCs at the 36-month recall period regarding marginal staining, where preheated RCs performed better. This could be attributed to the increased flowability of RCs upon preheating, enhancing marginal adaptation. These findings were confirmed by the results of Wagner and others, who concluded that the preheating procedure reduced microleakage and improved the adaptation of RC restoration to tooth structure.[31] Additionally, Yang and others proved that preheated RCs at 50°C for class I cavity preparations showed no microleakage at the tooth restoration interface, whereas nonheated RCs showed minor microleakage detected at occlusal margins.[32]

These findings also agree with Loguercio and others, who revealed 11% marginal staining in subjects after a 3-year follow-up when restored with nanofilled RCs using Single Bond Universal (3M ESPE) adhesives in the etch-and-rinse mode.[33] Yazici and others proved that a high incidence of marginal staining (14%) was evident in nanofilled RCs after a 3-year follow-up period when used in class I restorations.[23]

Regarding postoperative sensitivity, the majority of the cases scored 1 in relation to postoperative sensitivity. Thus, no difference was found between nonheated and preheated RCs, but significant differences were found regarding postoperative sensitivity over time in both groups.

These findings are in agreement with Campbell and others as they concluded that there is no detectable difference regarding postoperative sensitivity between room temperature and preheated RC restorations. That study also proved that when teeth were restored with RCs, postoperative sensitivity was significantly reduced from 24 hours after placement to that recorded after two weeks and that recorded one month later.[14] Additionally, these findings agree with Zanatta and others, who revealed 8.5% postoperative sensitivity in subjects at the baseline when restored with nanofilled RCs using Single Bond Universal adhesive in the etch-and-rinse mode.[34]

Moreover, these results observed postoperative sensitivity over a 36-month period and proved the significant decline of postoperative sensitivity over the whole review period as concluded by previous studies.[23,33,34] These secondary outcomes validate the sensitivity of the protocol used in the present study. Furthermore, a comprehensive literature review confirmed that this is the first attempt to measure postoperative sensitivity clinically using preheated RCs over a 36-month period.

Clinical data on the effect of the heating of RCs on the clinical performance of class I restorations are lacking, making comparison of our study results difficult. Thus, further studies are required to confirm our findings. In a literature review, one clinical trial evaluated the effect of composite preheating on postoperative sensitivity for 1 month.[14] Our study verified that there were no significant differences between the nonheated and preheated RCs for all the parameters, except for marginal staining. Hence, the null hypothesis stating that there is no significant difference in the clinical performance of preheated RCs in comparison with nonheated RCs for a 36-month follow-up period in class I restorations was partially rejected. One of the major limitations of this clinical investigation is that 36 months could be a short period for observing substantial changes. Thus, a long-term clinical evaluation may be able to better assess the effect of preheating nanofilled RCs.

CONCLUSIONS

After 36 months, the preheated nanofilled RCs showed acceptable clinical performance similar to that of the nonheated resin in class I restorations with better resistance to marginal staining.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and polices of the ethical committee of Mansoura University. The approval code issued for this study is A1810022136.MCEPRCRCL.

Conflict of Interest

The authors do not have any financial interests in the companies whose materials are included in this article.

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Does the Bleaching Gel Application Site Interfere With the Whitening Result? A Randomized Clinical Trial

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Clinical Relevance

Whitening gel can act at a distance, and its application on the total tooth is not necessary for an effective and homogeneous result. In addition, contact of the gel on cervical areas causes events of dental sensitivity.

SUMMARY

This study aimed to evaluate the effect of the bleaching gel application site on chromatic changes and postoperative sensitivity in teeth. Thirty patients were selected and allocated to three groups (n=10 per group), according to the location of the gel: GI, cervical application; GII, incisal application; and GIII, total facial. The amount and time of application of the 35% hydrogen peroxide (H_2O_2) gel were standardized. Color changes were analyzed by ΔE and W_{id} (bleaching index), using the values obtained in the readings conducted on a digital spectrophotometer in the cervical (CRs) and incisal regions (IRs) of the teeth. Spontaneous sensitivity was assessed using

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the questionnaire, and the stimulated sensitivity caused by the thermosensory analysis (TSA). The analysis occurred in five stages: baseline, after the first, second, and third whitening sessions (S), and 14 days after the end of the whitening, using the linear regression statistical model with mixed effects and post-test by orthogonal contrasts ($p<0.05$). Although the IR was momentarily favored, at the end of the treatment, the restriction of the application site provided results similar to those obtained when the gel was applied over the entire facial surface. Regarding sensitivity, only the GI showed spontaneous sensitivity. In the TSA, GIII had less influence on the threshold of the thermal sensation. It was concluded that the chromatic alteration does not depend on the gel application site. Spontaneous sensitivity is greater when the gel is concentrated in the cervical region (CR), and the teeth remain sensitized by thermal stimuli even after 14 days.

INTRODUCTION

Aligned teeth, presenting harmonious shapes with smile lines and facial proportions as well as with light tones, are part of patient desires for esthetics in dental treatment planning. The chromatic alterations of the unit or the entire dentition can be attenuated or even resolved using whitening techniques, which has been considered a low complexity procedure that is accessible to a large part of the population.[1,2]

Tooth whitening can be performed using the at-home technique in which the patient performs the treatment at home under the supervision of the dentist or using the in-office technique that is performed in the dental office. Both treatment options have hydrogen peroxide (H_2O_2) as an active ingredient; however, the peroxide concentration is generally considerably higher in the in-office technique as compared to the home technique.[3-5]

It is believed that for tooth whitening, H_2O_2 and other reactive oxygen species (ROS) present in the whitening gel diffuse rapidly through the dental tissues, seeking molecular stability when reacting with the chromophore agents. This reaction results in the continuous cleavage of the pigments until the saturation point occurs. This process is only possible because ROS are low in molecular weight and can permeate quickly through dental structures.[6-9]

Different hypotheses are being studied in order to better understand this treatment fully. Whitening can be considered a therapy in which an oxidizing substance is applied topically to the tooth enamel, as it is believed that it complies with Fick's second law, where the diffusion would be related to the residence time, the area of contact of the bleaching gel with the tooth surface, the volume of the product, and the thickness of the substrate.[10]

Regarding the area of application of the product, there are few clinical reports that point to a possible polydirectional action of peroxides, which could act on spots distant from the regions that received the product.[11-13] In 1990,[5] Haywood reported the possibility of the diffusion of H_2O_2 not only in the applied area but also in the entire tooth.

Despite this, when there is tooth staining, the professional often opts for the application of the product specifically on an affected area, with no intention of enhancing the whitening effect in the region and achieving chromatic uniformity in the tooth. The clinical results performed in patients with orthodontic brackets also indicate that the restriction of the contact area of the bleaching gel with enamel is of secondary importance, although, to date, controlled investigations that objectively evaluate this type of procedure have not been devised and developed.[14-16]

Thus, understanding the role of the bleaching gel application site in the chromatic alteration of different regions of the clinical crown can bring advances in whitening therapy and result in significant changes in the clinical protocols of in-office treatments. Therefore, the objective of this study was to evaluate the effect of the bleaching gel application site clinically on the chromatic alteration that occurred in different regions of the crown (cervical and incisal) and on the postoperative sensitivity reported by patients undergoing bleaching treatment in the office. The following null hypotheses were tested: 1) the gel application site would not influence the result of the crown whitening, and 2) the gel application site would not influence the postoperative sensitivity.

METHODS AND MATERIALS

Prior to this research, the project was submitted to the Research Ethics Committee. The study followed the CONSORT statement. Only after approval, clinical procedures were started.

Experimental Design

This was a randomized clinical study evaluating two factors: 1) Place of application of the gel on three levels: half-cervical, half-incisal, and total facial surface; and 2) Analysis times at five levels: baseline (T0), after the first (T1), second (T2), and third (T3) whitening session, as well as at 14 days (T4) after the end of the treatment. The experimental units evaluated were maxillary canines and the response variables were 1) Chromatic

alteration in the cervical part; 2) chromatic alteration in the incisal part, and 3) postoperative sensitivity.

Selection of Patients

The study included 30 volunteers of both sexes, aged between 18 and 30 years (average age 24.8 years). For the selection of patients, clinical and radiographic examinations were performed, as well as detailed anamnesis to verified whether the patients met the inclusion criteria (Table 1). The selected individuals were informed about the research, the possible risks, and the benefits obtained. In addition, patients were given instructions such as not taking analgesic or anti-inflammatory drugs and maintaining good oral hygiene and care, so that there would be no interference in the research results.

The sample calculation was performed based on a previous study,[17] using the software Sigma Plot 14.0. The test details were as follows: Significance level (α)=0.05; test power (1-β)=0.80; and dropout (β)=0.20. Thus, the minimal sample size required was 30 patients.

In the entire study, when considering elements 13 and 23 of these 30 patients, 60 dental elements were available for the study at random and independently.

Table 1: *Inclusion and Exclusion Criteria*

Inclusion Criteria
Patients who wanted to undergo whitening treatment
Patients with healthy and vital teeth 13 and 23
Patients with no carious or noncarious lesion
Patients without orthodontic appliances
Patients who have never performed whitening
Patients with good systemic conditions
Healthy oral soft tissue patients
Nonsmoking patients
Exclusion Criteria
Patients with indirect restorations in the teeth involved in the analysis
Patients who had a history of adverse reaction to peroxide
Patients who used opioids or drugs that influence the sensorineural response
Patients with tooth stains (tetracycline, trauma, fluorosis, and unknown etiology)
Patients with neurological diseases
Patients with chronic or acute diseases
Patients with exposed dental tissue
Patients with dental sensitivity or a history of treatment

Thus, it was possible to compare two techniques in the same patient, simultaneously, in order to reduce the influence of variables such as patient habits, environmental and habitual conditions of the oral cavity, and obtaining a minimum bias for the study (Table 1).

Randomization and Intervention

Randomization and dosage used in each arcade were determined by drawing lots. The possible combinations of treatments (Figure 1) for the study of gel application area were cervical application (CA) × total facial (TB) and incisal application (IA) × total facial (TB).

These combinations were recorded on 30 cards, contained in sealed, opaque envelopes, and numbered sequentially. These were drawn by a team member not directly involved in the study. The group allocation was revealed when opening the envelope on the day of the bleaching procedure.

Whitening Therapy

The volume of the bleaching gel used on each tooth was standardized, using the information provided by the manufacturer as a parameter. Thus, as each bleaching gel syringe contains 5 g and is intended for four bleaching sessions with 20 teeth in each, each tooth received the amount of 0.06 g per session.

The patients received treatment using the office whitening technique, which used the whiteness HP AutoMixx whitening product (FGM Dental Products, Joinville, Santa Catarina, Brazil) composed of 35% H_2O_2, using no source of physical activation.

Initially, dental prophylaxis was performed with a rubber cup and a paste, obtained by mixing pumice and water, moving at a low speed. Subsequently, the

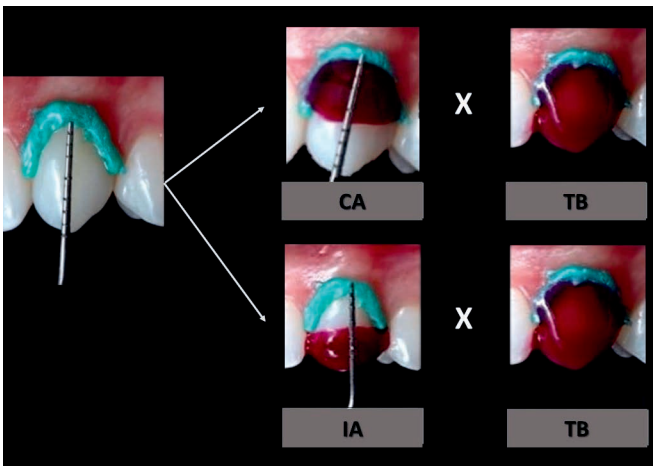

Figure 1. *Combinations of whitening treatments (CR × TV; IR × TV).*

oral soft tissues were isolated with a photoactivated TopDam (FGM) gingival barrier.

The bleaching product used was provided in double-body syringes, with peroxide and thickening agents in separate compartments. Through a self-mixing tip, the resulting gel was provided in pipettes for viscous liquids, (Microman E Gilson, West Beltline, Hwy Middleton – EUA) being applied as 0.06 g of bleaching product on each tooth, according to the proposed design. The bleaching gel remained in the area of interest for 45 minutes. After 7 and 14 days, the procedures were repeated.

The remaining teeth received the whitening therapy in a conventional way, after completing the three whitening sessions and the follow-up period.

Analysis Times

The analysis was performed at the pre-established study times: T0, baseline; T1, after the first whitening session; T2, after the second whitening session; T3, after the third whitening session; and T4, 14 days after the end of the whitening therapy.

Digital Analysis of Color Change

The study of the chromatic alteration occurring in the tooth was carried out with the digital spectrophotometer, Vita Easyshade Advance (Vita Zahnfabrik, Bad Säckingen, Germany). In order to standardize the reading area, initially, patient impressions were cast, and plaster models were obtained for later making two acetate trays on each model—one containing a perforation in the cervical region (CR) and the other intended for reading in the incisal region (IR). Regardless of the treatment received for each tooth for research (teeth #6 and #11), measurements were made on both the cervical and incisal portions of the teeth. Thus, the acetate trays with their perforations enabled the standardized positioning of the tip of the portable spectrophotometer in order to perform the measurement always in the same region of interest, according to the previously established times.

The perforations made it possible to standardize the Easyshade using the CIE $L^*a^*b^*$ color model, established by the Commission International de I'Eclairage—CIE (International Commission on Lighting), which allows the specification of color perceptions in three-dimensional models. The axial "L" is known as the luminosity and extends from 0 (black) to 100 (perfect white). The "a" coordinate represents the amount of red (positive values) and green (negative values), while the "b" coordinate represents the amount of yellow (positive values) and blue (negative values).

The color readings were performed on the incisal half and cervical half of teeth #6 and #11, respecting the pre-established compared times with the initial reading, through the calculation below:

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

Whitening Index (W_{id})

To verify the perception of tooth whitening, the whitening index (W_{id}) was also calculated. The W_{id} is a simple, linear formulation that uses values of the chromatic coordinates of CIELab, and the higher the values obtained by the calculation the greater the lightening effect provided by a given procedure. *The whitening index* was calculated using the following formula[18]:

$$W_{id} = (0.511 \times L^*) - (2.324 \times a^*) - (1.100 \times b^*)$$

Sensitivity Analysis

Spontaneous Sensitivity—Visual Analog Analysis (VAS)— Dental sensitivity was assessed daily. The patients themselves reported the presence or absence and the intensity of sensitivity in the period between the whitening sessions. The highest score recorded in the period under analysis was considered for the comparison of the groups under study. The patients were asked about the location (tooth #6 or #11) and the intensity of the discomfort caused by the treatment, with a value of 0 for patients who did not present with any painful symptoms and 10 when severe sensitivity occurred.[19,20]

Analysis of Thermal Sensation Threshold—TSA II— For this analysis, another acetate tray was made, now containing a central perforation in order to standardize the location of the thermal stimulus positioning. For that, the TSA-II equipment (Medoc Advanced Medical Systems Ltd, Ramat Yishai, Israel) was used, which has an intraoral thermal mode that was always positioned in the same region of the crown. Prior to its use, 0.02 mL of thermally conductive paste, composed of silver oxide, was applied to the enamel to ensure that the thermal stimulus generated by the equipment reached the same area of the crown. This test was carried out at the previously established times.

To check the sensation threshold, the TSA II equipment was configured in the "Limits" function, which performs three tests with falling temperatures (from 36°C to 0°C). Each test starts at 36°C, and the thermal mode temperature is gradually reduced at a speed of 1°C/second. When the thermal stimulus is perceived by the patient, it interrupts the stimulus, and the temperature is registered in the software that

manages the production of the thermal stimuli. Thus, the lower the recorded temperature, the less sensitive the tooth was.[21,22,19]

Statistical Analysis

After tabulation, descriptive, and exploratory analysis of the data, compliance with the assumptions of normality and homogeneity with the SAS 9.4 software was found. Then, the data were submitted to the linear regression model with mixed effects (random and fixed effects). For comparisons, the post-test using orthogonal contrasts was used. All tests adopted a significance level of 5%.

RESULTS

Demographic Characteristics and Adherence

After evaluating 52 patients, 30 volunteers met the inclusion criteria. The allocation of patients was described using the CONSORT flowchart (Figure 2). Table 2 shows the demographic characteristics of the volunteers.

Chromatic Change Analysis—ΔE and W_{id}

Table 3 shows that, when comparing the results obtained with the application of the product in the CR with those throughout the crown, except for the second whitening session, the chromatic alteration (ΔE) of the cervical and the IRs were always similar. When analyzing the evolution of treatment over time, both the CR and the IR had a progressive increase in ΔE. Note that the IRs were saturated in the second whitening session, while, in the CR, the biggest changes were observed after the third session, regardless of the location of the gel application. It was also observed that the CR always presented chromatic recurrence, while the IR only relapsed when the gel was applied to the CR. The application site did not influence the cervical or incisal chromatic alteration in any analyzed time.

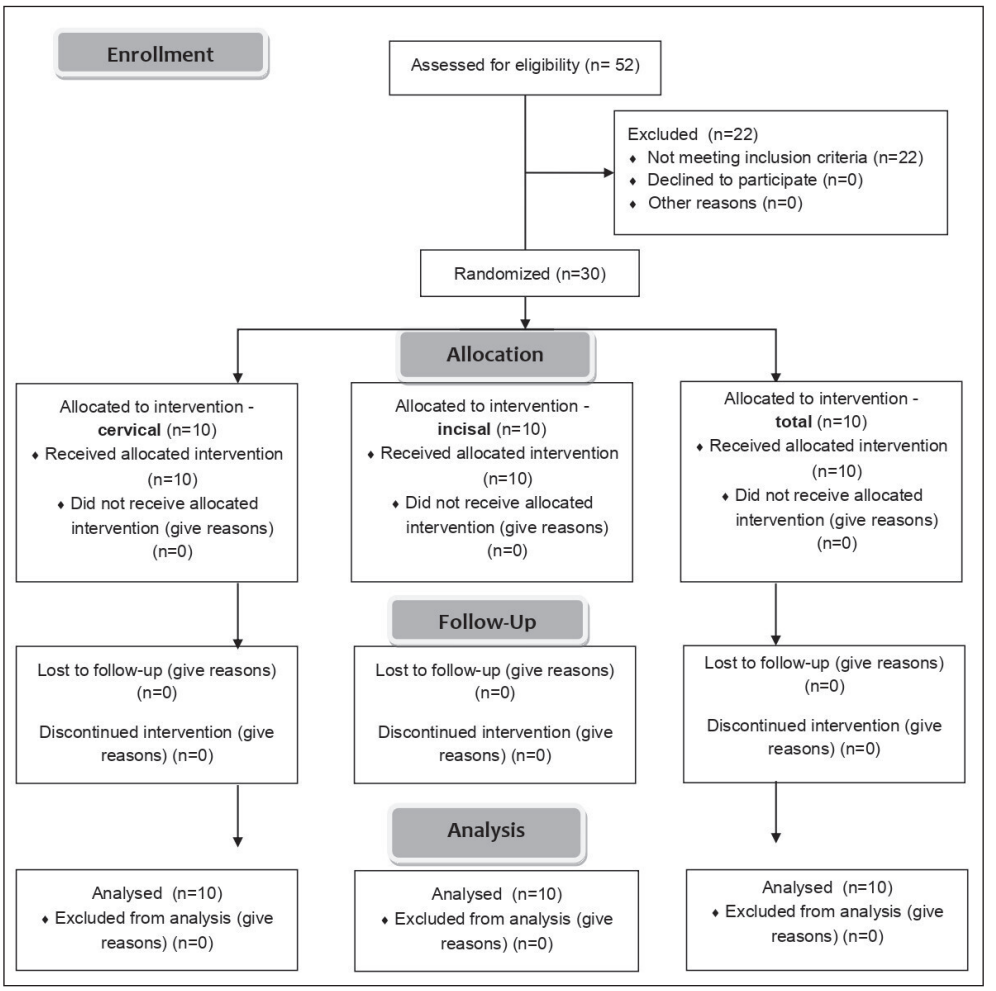

Figure 2. *CONSORT flowchart with allocation, monitoring, and analysis during the study.*

Table 2: *Demographic Characteristics of Patients*		
Age	**24.8 (±3.68)**	
Sex (%)	Female	66.66%
	Male	33.33%
Ethnic-racial (%)	White	50%
	Brown	23.33%
	Black	26.66%

Table 3: *Averages of ΔE and W_{id} Values in Different Reading Regions (CR) and (IR), with Application of the Whitening Gel in (CA) and (TB), at Different Times Analyzed*[a]

Cervical x Total Facial (TB) application									
CA		**1 Session**		**2 Session**		**3 Session**		**14 days**	
		TB	**CA**	**TB**	**CA**	**TB**	**CA**	**TB**	**CA**
ΔE	CR	8.9 Ab	6.8 Ac	9.8 Abc	9.0 Bb	14.5 Aa	12.7 Aa	11.7 Ab	10.3 Ab
	IR	7.9 Ac	8.5 Ab	12.0 Aab	12.0 Aa	14.8 Aa	13.7 Aa	11.9 Ab	11.9 Aa
W_{id}	CR	8.9 Ac	8.0 Bc	11.8 Abc	11.5 Bb	17.8 Ba	17.3 Ba	14.6 Ab	14.6 Bb
	IR	11.5 Ac	10.6 Ac	15.4 Aab	14.4 Ab	20.9 Aa	20.5 Aa	17.2 Ab	16.2 Aab

[a] *Uppercase letters compare lines in each application area. Lowercase letters compare columns (time). Asterisk compares the application area at the same reading location and at the same time.*

Regarding the whitening index, it can be observed that, in general, the changes produced in the region were more pronounced in the IR than those observed in the CR, especially when the gel was applied across the crown. When the gel was applied to the CR, the whitening effect was similar in both the regions (in the first and second sessions, and 14 days after). An analysis of the evolution of the whitening effect showed that the best results were found after a third whitening session, with significant recurrence at 14 days. The application area (in the cervical or whole crown) did not influence any lightening effect in any analyzed time or region (Tables 3 and 4).

Table 3 shows the averages of Δ*E* and W_{Id} values in different reading regions (CR) and (IR), with the application of the whitening gel in (CA) and (TB), at different times analyzed. Table 4 shows the values of Δ*E* and W_{id} obtained in different regions of the crown when the application was carried out in the IA or in the total facial (TB) region.

When comparing the Δ*E* values in the different regions, it was found that in the first and second whitening session, the IR showed more pronounced changes than the cervical, when the gel was applied to the IR. However, in the third session as well as 14 days later, the incisal and CRs provided similar values of Δ*E*, regardless of the application site. It was also found that throughout the treatment the IR showed chromatic stabilization after the second session, while the CR continued to show an increase in Δ*E* values until the third whitening session. Chromatic recurrence did not occur in the IR when the gel was applied in the same region and in the CR when the gel was applied to the entire crown. When comparing the effect of the gel application site on the chromatic alteration (IA × TB), it was observed that the incisal application provided higher values of Δ*E* in this region in the second and third sessions but returned to be similar after 14 days. In the CR, they were always similar.

Regarding the whitening index, it was found that the changes produced in the IR were similar to those observed in the CR, when the gel was applied in the IA, except after the third whitening session in which the IR presented the largest change. When evaluating the whitening effect when the gel was applied TB, the changes produced in the IR were more pronounced than those observed in the CR at all the times analyzed. The analysis of the evolution of the whitening effect showed that the IR was stabilized after the second session, when the gel was applied to the same area. However, when the gel was spread throughout the crown, the IR continued to be cleared until the third session. The CR always showed the greatest whitening effect in the third session. It was also observed that both the cervical and IRs showed a decrease in the W_{id} after 14 days. The effect of the application site was not significant in the

Table 4: *Averages of ΔE and W_{id} Values in Different Reading Regions (CR) and (IR), with Application of the Whitening Gel in (IA) and (TB), at Different Times Analyzed*[a]

Incisal × Total Facial (TB) Application									
IA		**1 Session**		**2 Session**		**3 Session**		**14 Days**	
		TB	**IA**	**TB**	**IA**	**TB**	**IA**	**TB**	**CA**
ΔE	CR	7.1 Bc	6.8 Ac	9.5 Bb	9.0 Bbc	13.1 Aa	12.7 Aa	10.5 Ab	10.3 Aab
	IR	9.2 Ab	8.5 Ac	13.6 Aa*	12.0 Aab	15.4 Aa*	13.7 Aa	12.8 Aa	11.9 Ab
W_{id}	CR	8.9 Ac	8.0 Bc	11.8 Abc	11.5 Bb	17.8 Ba	17.3 Ba	14.6 Ab	14.6 Bb
	IR	11.5 Ac	10.6 Ac	15.4 Aab	14.4 Ab	20.9 Aa	20.5 Aa	17.2 Ab	16.2 Aab

[a] *Uppercase letters compare lines in each application area. Lowercase letters compare columns (time). Asterisk compares the application area at the same reading location and at the same time.*

W_{id} values at any analyzed time or in any region (Table 4 and Figure 3).

Analysis of Postoperative Sensitivity

Absolute Risk and Spontaneous Sensitivity—When analyzing the absolute risk of sensitivity, it was observed that only the group in which the gel was restricted in the CR generated spontaneous sensitivity, which affected 15% of patients after the second session and 10% after the third whitening session (Table 5). At these times, the maximum intensity reported by patients was a score of 5.

Assessment of Neurosensory Analysis—TSA II—Figure 4 shows that after the first session all forms of application provided a similar effect on the sensitive threshold of canines. The average cold detection temperature ranged between 8.7°C and 10.3°C. However, after the second and third sessions, and even after 14 days, the application of the gel across the crown was the form that left the teeth less sensitive to cold stimuli and was still similar to the results obtained when the application was at the IR. It was also observed that when the application of the gel was in the CR, the sensory threshold was changed until the third whitening session, while when the application was in the IR or in the entire crown, the thermal stimulus that caused the discomfort grew only until the second session. Despite this, in all forms of application, the teeth remained sensitized after 14 days.

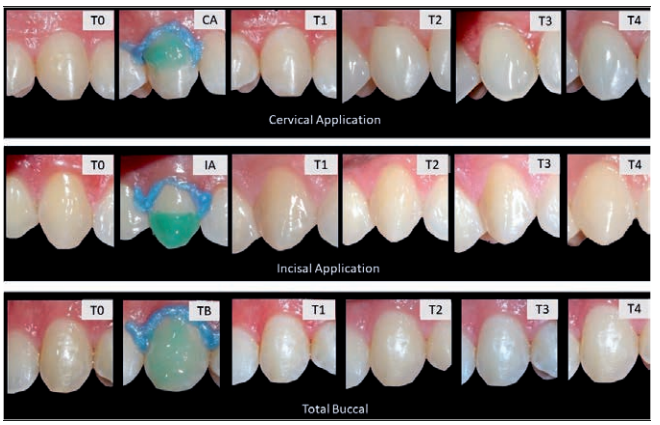

Figure 3. *Chromatic change in different applications in the previously established operative times.*

DISCUSSION

Despite the knowledge of the permeability of dental structures to whitening agents, most professionals extend the area of application of the whitening gel to the maximum extent—both in the home and office techniques.[3-5] It is also very common among professionals to deposit the gel in darker areas, believing that the application site plays a decisive role in the success of whitening therapy.[14,15] This approach can expose the patient to accidental contact of the gel with the gingival tissue, generating mild burns, which decreases the feeling of satisfaction with the treatment.[23,24]

In this context, one of the objectives of this study was to evaluate whether the application of the whitening product in the CR or IR would provide different results compared to those obtained when the same volume of gel is applied throughout the crown. Δ*E* values showed that although the IR may be momentarily favored by the application of the gel directly in this region, at the end of the session as well as 14 days after the end of the treatment, the restriction of the application site of the gel in the cervical or IR provided results similar to those obtained when the gel was applied across the crown; therefore, the first hypothesis was accepted.

This result shows that the diffusion of peroxide and other reactive oxygen substances occurs quickly and in a polydirectional manner and does not depend exclusively on the main orientation of the expressed diffusion pathways, formed by the porosities in the

Table 5: *Absolute Risk and Maximum Intensity of Sensitivity at the Different Analyzed Times*[a]

	After the 1st Session		After the 2st Session		After the 3st Session		14 Days After Treatment	
	Absolute Risk	Maximum Intensity	Absolute Risk	Maximum Intensity	Absolute Risk	Maximum Intensity	Absolute Risk	Maximum Intensity
CA	0% Ab	0 Ab	15% Aa	5 Aa	10% Aa	5 Aa	0% Ab	0 Ab
IA	0% Aa	0 Aa	0% Ba	0 Ba	0% Ba	0 Ba	0% Aa	0 Aa
TB	0% Aa	0 Aa	0% Ba	0 Ba	0% Ba	0 Ba	0% Aa	0 Aa

[a] *Uppercase letters compare lines in each application area. Lowercase letters compare columns (time).*

interprismatic region, in the nuclei of the prisms, and the dentinal tubules. This is because the reactive oxygen substances are low-molecular-weight molecules that have the ability to pass through the intertubular secondary pathways, allowing regions that have not received the gel to show significant chromatic changes, at least when the whitening treatment produces a large amount of ROS, like in the in-office technique.[6,7,25,26]

The penetration of H_2O_2 is only possible because the enamel is a semipermeable structure formed by prisms and a sheath rich in proteins of approximately 26 nm, directing and modulating the intensity of the diffusion of ROS through interprismatic and intercrystalline spaces.[26,27,28,29] Once in the dentinal tissue, ROS travel easily through the tubules towards the pulp chamber, because the tubules have an increasing diameter and density in relation to the pulp. In addition, they have canaliculi that promote intertubular communication, which explains the bleaching action far from where the gel is applied. Thus, when running through the entire structure of the tooth, reactive oxygen species from the whitening product interact with the chromophore molecules present in the dental structures, cleaving them, increasing the luminosity of the tooth, and decreasing its chroma.[3,4,28,30]

As a complementary analysis, the evolution of treatments over time was analyzed, noting that the IR had already saturated in the second whitening session, while the CR achieved the greatest variations in chromatic alteration after the third session. It is known that H_2O_2 and other reactive radicals have a very short useful life and, as a consequence, it can be assumed that the layers closest to the bleaching product respond more quickly to whitening than the dentin.[31] Thus, it can be inferred that the IR, being thinner, responds more quickly to treatment. In addition, Ma in 2011[32] and Vieira in 2008[33] stated that during whitening the enamel can increase the amount of water present in the structure, which makes it the most luminous enamel with the least visibility of the underlying dentin.[34-36]

It is worth mentioning that in addition to the chromatic alteration evaluated by ΔE, the result of the W_{id} bleaching was also analyzed, which allowed the analysis of the bleaching of the structures. This

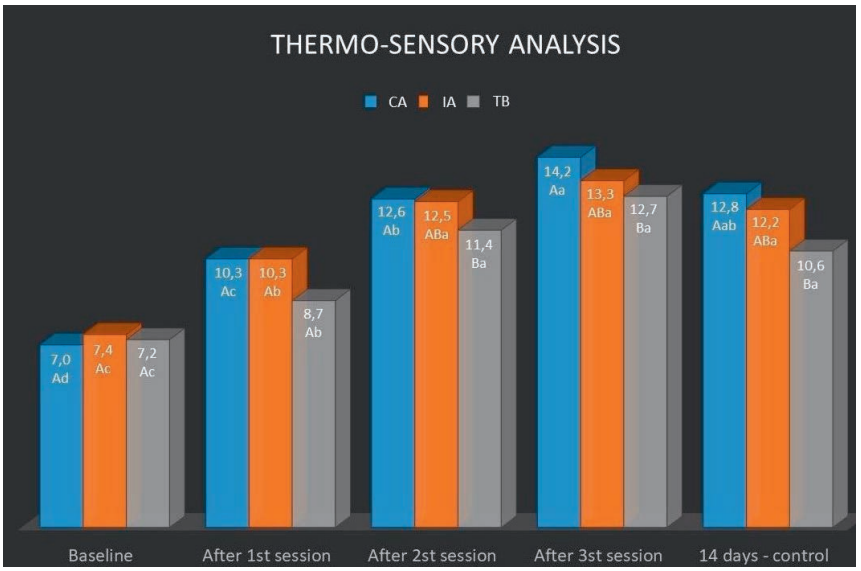

Figure 4: *Averages of the values of the thermosensory analysis (TSA) for each group in the analyzed times.*

index allows an evaluation through the correlation of visual perception and the coordinates of the CIELab system. Thus, it was possible to observe that the greatest whitening effect occurred in the IR, especially when that region received the whitening gel. This can be explained by the fact that the middle- and cervical-thirds have a greater thickness and opacity, as they have a pulp chamber filled with connective tissue as a background, which can limit the lightening effect of the region.[37,38]

Although some research has shown that whitening saturation can be achieved in the second session,[39-41] in the present study, the highest values of the whitening index were observed after the third session in all the variations of application area present in the study design, with standardization of time and volume of bleaching gel. These results are probably related to the teeth used in this work, that is, canines, which are darker and more opaque than the maxillary-central incisors, which are generally used in clinical research to measure the lightening effect.[31,42]

The results observed in the present study confirm the polydirectional whitening action—a fact already explored in previously published clinical case reports. In this context, Gomes and others[14] showed that the whitening treatment performed on patients with orthodontic accessories showed no difference from the results observed when the gel was applied to the entire crown.

Another objective of the study was to analyze the influence of the gel application site on tooth sensitivity. Spontaneous sensitivity analysis detected the greatest propensity for sensitivity events when the gel was applied in the CR after the second and third whitening sessions. Possibly, the diminished enamel thickness in this region favored the spread of ROS speed to the pulp–dentin complex. It is known that the penetration of H_2O_2 into the pulp results in the release of biochemical mediators involved in the inflammatory process, which sensitizes pulp nociceptors, changing the sensitivity threshold of nerve fibers and obtaining spontaneous sensitivity reports.[43-45]

Despite this, the occurrence of sensitivity was very low in relation to that reported in other studies, in which the treatment was carried out in the entire arch, using various dental groups with nonindividualized volumes of gel, which resulted in a higher occurrence of sensitivity, mainly in the lower and upper incisors. [20,46-49]

Regarding postbleaching sensitivity, it was found that the sensitivity to thermal stimuli gradually increased until the third whitening session, and that the teeth remained sensitized until 14 days after the end of treatment. The permanence of dental sensitization was also reported by Rahal and others,[19] who associated this phenomenon with the activation of the TRPA1 ion channel, generating an inflammation process and subsequent stimulation of precipitated thermal sensation—the result of a possible reversible inflammation in pulp tissue. In addition, events of histomorphological changes in the dental enamel, through the direct action of the whitening agent on the proteins present in the tooth and an increase of the tissue's diffusion and permeability channels, can directly affect the influence of the response to the thermal stimulus, making the tooth more susceptible to the thermal sensation when subjected to a cold stimulus.[19,44,50] Thus, the results showed that spontaneous and provoked sensitivity varied according to the product's application protocol, and, therefore, the second hypothesis of the study was rejected.

Thus, it was observed that the diffusion capacity of ROS resulted in a chromatic alteration in all the evaluated regions, regardless of the place of application of the gel. In addition, the application restricted to the CR had a negative influence on the sensory response. Therefore, new studies are suggested that address the influence of the area of application of the whitening gel as well as the incorporation of new variables of direct influence in the response of the whitening therapy, such as the volume of the whitening gel and the influence of the anatomy of different teeth.

CONCLUSIONS

- The chromatic changes of the CRs and IRs do not depend on the place of application of the bleaching gel.
- The IR reaches chromatic saturation faster than the CR.
- The restricted application of the bleaching gel in the CR left the teeth more sensitive.
- Despite the remission of spontaneous symptoms, all groups remained sensitized to low temperature 14 days after the end of treatment.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies of the Research Ethics Committee FOA/UNESP. The approval code issued for this study is 91141018.6.0000.5420.

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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Five-year Randomized Clinical Trial on the Performance of Two Etch-and-rinse Adhesives in Noncarious Cervical Lesions

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Clinical Relevance

The two adhesive systems tested, a polyalkenoic acid-containing adhesive or an MDP-containing adhesive, had comparable clinical performance, at 60 months, when used to restore noncarious cervical lesions.

SUMMARY

Objectives: To evaluate the 5-year clinical performance of two-step etch-and-rinse adhesives in noncarious cervical lesions (NCCL).

Methods and Materials: The sample comprised 35 adults with at least two similar-sized NCCL. Seventy restorations were placed, according to one of the following groups: Adper Single Bond 2 (SB) and Ambar (AM). The restorations were placed incrementally using a resin composite (Opallis). The restorations were evaluated at baseline and after 6 and 18 months and 5 years using some items of the FDI criteria. The differences in the ratings of the two materials after 6 months, 18 months, and 5 years were performed with Friedman repeated measures ANOVA by rank and McNemar test for significance in each pair (α=0.05).

Results: Five patients did not attend the 60-month recall. No significant differences were observed between the materials for any criteria evaluated.

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Twenty-one restorations failed (12 for SB and 9 for AM) after 60 months. Thus, the retention rate for SB at 60 months were 55.6% for SB and 71% for AM (p=0.32). After 60 months, 12 restorations (6 for SB and 6 AM) showed some loss of marginal adaptation (p=1.0). Slight marginal discoloration was observed in 10 restorations (6 for SB and 4 AM; p=0.91). Five restorations (2 for SB and 3 for AM) showed recurrences of caries (p=1.0).

Conclusions: Both two-step etch-and-rinse adhesives—Adper Single Bond 2, a polyalkenoic acid-containing adhesive, and Ambar, a 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-containing adhesive—showed acceptable clinical performance after 60 months.

INTRODUCTION

A noncarious cervical lesion (NCCL)—a frequent and challenging condition to treat—is described as the loss of hard tooth tissue at the cementoenamel junction.[1] Data from the literature regarding prevalence of NCCLs show a high prevalence of NCCLs, ranging from 35.4%[2] up to 77.3%.[3] Besides compromising esthetics and function, up to 92.1% of NCCLs result in dentin hypersensitivity, as reported in the systematic review of prevalence studies.[4] A restorative procedure is the main way to reestablish the lost dental substrate and minimize dental sensitivity.[5]

Unfortunately, NCCLs are difficult to restore, because the margins of these lesions are located in the cementum or dentin, jeopardizing moisture control and access to the gingival margins.[6] Furthermore, they present a high index of sclerotic dentin,[7,8] which reduces bonding efficacy when compared to sound dentin.[7] Due to these adverse conditions, NCCLs are the best model to test the clinical effectiveness of adhesives.[9] Additionally, due to the presence of NCCLs in several teeth of the same patient, it is easy to compare adhesive systems in a split-mouth study design.[9,10]

Although several adhesive strategies have been developed, one of the most used currently is the application of phosphoric acid associated with an adhesive system. This technique was launched in the mid-1980s,[11] and it was originally called "total-etch," because the enamel and dentin are etched simultaneously with phosphoric acid.[12,13] However, the term "etch-and-rinse" has been used, because it better represents the technical procedure.[14] The etch-and-rinse strategy is divided according to the number of bottles in a two-step or three-step system.[11] After etch-and-rinse, hydrophilic and solvent-based adhesives are applied, and are responsible for infiltration into the demineralizing dentin. Afterward, the polymerization is performed, and the adhesive becomes micromechanically bonded into dentin to form the hybrid layer.[15] However, in the etch-and-rinse adhesives, the micromechanical interlocking is a prerequisite for achieving a strong mechanical bond.[10]

Actually, the addition of functional monomers with the potential capacity for chemical adhesion to the tooth structure could be beneficial in terms of durability, because it ensures an intimate adaptation of the substrate and biomaterial components, thereby preventing nanoleakage.[16] Two monomers with this chemical potential are polyalkenoic acid copolymer (PAC) and 10-methacryloxydecyl dihydrogen phosphate (MDP).[11] The former was first used in the composition of Vitrebond (3M Oral Care) and more recently has been used in several adhesive formulations from the same manufacturer.[17,18] Due to PAC's ability to chemically bond to the calcium in hydroxyapatite, a good clinical performance has been observed when PAC-containing etch-and-rinse adhesives are used.[19-21] On the other hand, it is well documented that, due to the formation of highly hydrolytically stable MDP-Ca salts,[22] the presence of MDP promotes a stable chemical bond with dental substrates.[22] Despite only recent use, the clinical performance of MDP-containing etch-and-rinse adhesives has been evaluated and has shown good results.[23-25]

However, to the extent of the author's knowledge, only a short-term (18-month) randomized clinical trial was found that compared an adhesive containing-PAC versus an adhesive containing-MDP, with similar results between both the materials.[26] Thus, the objective of this randomized clinical trial was to compare the 5-year failure rate of an adhesive-containing PAC versus an adhesive-containing MDP, with both applied in the etch-and-rinse mode, in a paired-tooth study design. The null hypothesis was that the failure rate of the composite restorations were placed with both the adhesive systems will be same after 60 months of clinical service.

METHODS AND MATERIALS

Study Design

This was a randomized, double-blind clinical trial, and it was described following the Consolidated Standards of Reporting Trials (CONSORT) statement.[27] The restorations were placed in the clinic of the School of Dentistry at the local university from July 2010 to July 2011. All participants were informed about the nature and objectives of the study, but they were not aware of which tooth received the specific treatments under evaluation.

Participant Recruitment

Written informed consent was obtained from all participants prior to starting the treatment. A total of 51 participants were examined by two calibrated dentists to check if the subjects met the inclusion and exclusion criteria. The evaluations were performed using a mouth mirror, an explorer, and a periodontal probe.

The inclusion criteria were the following: participants between 20 and 70 years old had to be in good general health, have an acceptable oral hygiene level, and present at least 20 teeth under occlusion. Participants were required to have at least two NCCLs to be restored in two different teeth. These lesions had to be noncarious, nonretentive, and deeper than 1 mm, and had to involve both the enamel and dentin of vital teeth without mobility. The cavosurface margin could not involve more that 50% of enamel.[20,21] All the patients were given oral hygiene instructions before the operative treatment was performed. Patients with extremely poor oral hygiene, severe or chronic periodontitis, or more than two wear facets on the occlusal surface of posterior teeth were excluded from the study.

Sample Size Calculation

The sample size calculation was based on the failure rate of the predecessor of the Adper Single Bond 2 (SB) (Adper Single Bond; 3M Oral Care, St. Paul, MN, USA; also known as Single Bond, Scotchbond 1 and Adper Scotchbond 1 in some countries) reported in earlier studies.[19-21] Using an α of 0.05, a power of 90%, and a equivalence limit of 20%, a minimum of 35 participants with two similar-sized NCCL were required. Taking that into consideration, 51 participants were evaluated, 15 subjects were excluded.

Randomization and Allocation Concealment

The randomization process was performed (using software available at http://www.sealedenvelope.com) by a staff member not involved in the research protocol. The allocated group's details were recorded on cards in sequentially numbered, opaque, and sealed envelopes. These were prepared by a staff member not involved in any of the clinical trial phases. The allocation assignments were revealed by opening the envelope immediately before the restorative procedure to guarantee the concealment of the random sequence and prevent selection bias. The allocation assignment was revealed by opening the envelope on the day of the restorative procedure, which ensured the concealment of the random sequence. In all cases, the tooth with the highest FDI tooth number received the treatment described first, while the tooth with the next number in sequence received the treatment mentioned second. The participants and the examiners were blinded to the group assignments.

Restorative Procedure

All of the patients selected for this study received dental prophylaxis with a suspension of pumice and water in a rubber cup, and signed an informed consent form two weeks before the restorative procedures were initiated.

The degree of sclerotic dentin from the NCCLs was measured according to the criteria described by Swift and others (Table 1).[28] The cavity dimensions in millimeters (height, width, and depth), the geometry of the cavity (evaluated by profile photograph and labeled at <45°, 45°-90°, 90°-135°, and >135°), the presence of an antagonist, and the presence of attrition facets, the distribution of enamel in the cervical margin was observed and recorded. Preoperative sensitivity was also evaluated by applying air for 10 seconds from a dental syringe placed 2 cm from the tooth surface and with an explorer. These features were recorded to allow comparison of the baseline features of the dentin cavities among experimental groups.

In order to calibrate the restoration procedure, the study director placed one restoration of each group in order to identify all steps involved in the application technique. Then the two operators, who were resident dentists with more than 4 years of clinical experience in operative dentistry, placed four restorations, two in each

Table 1: *Dentin Sclerosis Scale*[a]

Category	Criteria
1	No sclerosis present; dentin is light yellowish or whitish, with little discoloration; dentin is opaque, with little translucency or transparency
2	More sclerosis than in category 1 but less than halfway between categories 1 and 4
3	Less sclerosis than in category 4 but more than halfway between categories 1 and 4
4	Significant sclerosis present; dentin is dark yellow or even discolored (brownish); glassy appearance, with significant translucency or transparency evident

[a] *Adapted from Swift and others,[39] with permission from Elsevier.*

group, under the supervision of the study director in a clinical setting. The restoration failures were shown to the operators prior to starting the study. At this point, the operators were considered calibrated to perform the restorative procedures.

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

Before the restorative procedures, the operators anesthetized the teeth with a 3% mepivacaine solution (Mepisv, Nova DFL, Rio de Janeiro, RJ, Brazil), and cleaned all lesions with pumice and water in a rubber cup, followed by rinsing and drying. Then shade selection was made using a shade guide Vita Classical (VITA Zahnfabrik, Bad Säckingen, Germany). Following the guidelines of the American Dental Association (ADA),[29] no additional retention or bevel was prepared.

A rubber dam was placed, and then the NCCLs received the Adper Single Bond 2 (3M Oral Care; also known as Single Bond 2, Adper Single Bond Plus and Adper Scotchbond 1XT in some countries) or Ambar (FGM, Joinville, SC, Brazil) adhesive system, which defined the two different groups. The compositions and application modes are described in Table 2.

Both adhesives were applied according to the manufacturer's instructions (Table 2). Briefly, the cavity was etched with 37% phosphoric acid (CondAc 37, FGM) for 15 seconds, then rinsed with water for 15 seconds, and gently dried with an oil-free air stream, leaving the dentin surface slightly moist. The adhesive was scrubbed for 10 seconds on the cavity surfaces, and the solvent was evaporated with an air stream for 20 seconds. Another coat of adhesive was applied for 10 seconds, the solvent was evaporated for 20 seconds, and the adhesive layer was light cured (Radii-Cal, SDI, Victoria, Australia) for 10 seconds at 1200 mW/cm^2.

Two or four increments of resin composite (Opallis, FGM) with less than 2 mm were placed, and each one was light cured for 40 seconds. Finally, the restorations were finished and polished using fine-grit diamond burs (#3195F and #3195FF, KG Sorensen, Barueri, São Paulo, Brazil.) and flexible abrasive disks (Diamond Pro, FGM).

Clinical Evaluation

Two experienced and calibrated dentists, not involved with the restoration procedures and therefore blinded to the group assignment, performed the evaluations. For training purposes, the examiners observed 10 photographs that were representative of each score for each criterion. They evaluated from 10 to 15 patients each on two consecutive days. These subjects had cervical restorations but were not part of this project. An

Table 2: *Materials, Compositions, and Application Mode*

Materials (Batch Number)	Compositions	Application Mode[a]
Adper Single Bond 2	Acid: phosphoric acid 37% Adhesive: bisphenol glycidyl dimethacrylate, hydroxyethyl methacrylate, dimethacrylates, polyalkenoic acid copolymer (PAC), initiators, water, ethanol	Acid etch for 15 seconds; Rinse with water for 15 seconds; Dry the tooth surfaces for 5 seconds, but avoid excessive drying of the dentin; Apply one coat of adhesive system under vigorous agitation for 10 seconds; Evaporate the solvent for 20 seconds; Apply a second coat of adhesive system under vigorous agitation for 10 seconds; Evaporate the solvent for 20 seconds; Light cure for 10 seconds;
Ambar	Acid: 37% silica-thickened phosphoric acid gel Adhesive: 10-methaclyloxydecyl dihydrogen phosphate, urethane dimethacrylate (UDMA), 2-hydroxyethyl methacrylate, and other hydrophilic and acid methacrylate monomers, ethanol, silanated silica, photo-initiators, co-initiators, and stabilizers	1-8 (Same as for Adper Single Bond 2)

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

intraexaminer and interexaminer agreement of at least 85% was necessary before beginning the evaluation.[21,22] After recording the parameters during evaluation using a standardized paper case report form, the evaluation paper had to be sent back to the research staff, so that evaluators were blinded to group assignment during follow-up recalls.

The restorations were evaluated by FDI[30] criteria (Table 3) at baseline and after 6, 12, 18 and 60 months of clinical service. Only the clinically relevant measures for evaluation of the performance of adhesives were used and scored (Table 3). The primary clinical outcome was restoration retention/fractures, but the following secondary outcomes were also evaluated: marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries. The evaluation of the spontaneous postoperative sensitivity was performed 1 week after the restorative procedure. These variables were ranked according to the FDI criteria in the following scores: VG = clinically very good; GO = clinically good; SS = clinically sufficient/satisfactory; UN = clinically unsatisfactory; PO = clinically poor.

Both the examiners evaluated all of the restorations once and independently. When disagreements occurred during the evaluations, they had to reach a consensus before the participant was dismissed. The restoration retention rates were calculated according to the ADA guidelines[29]: Cumulative failure percentage = $[(PF + NF)/(PF + RR)] \times 100\%$, where PF is the number of previous failures before the current recall, NF is the number of new failures during the current recall, and RR is the number of currently recalled restorations.

Statistical Analysis

The statistical analyses followed the intention-to-treat protocol according to the CONSORT (Consolidated Standards of Reporting Trials) suggestion.[27] Descriptive statistics were used to describe the distributions of the evaluated criteria. For all outcomes (retention/fracture, marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries), the differences between the two groups' ratings after 60 months were tested by Friedman's repeated measures analysis of variance rank (α=0.05). Cohen's kappa statistics were used to test the interexaminer agreement (α=0.05) (Statistica for Windows 7.0, StatSoft Inc., Tulsa, OK, USA).

RESULTS

Thirty-five subjects (18 male and 17 female), with a mean age of 45 years, were enrolled in this study. Seventy restorations were placed (35 for each group). All baseline details relative to the research subjects and characteristics of the restored lesions are displayed in Table 4.

The overall Cohen's Kappa statistics (0.87) showed good agreement between the examiners. All research subjects were evaluated at baseline and the 6-, 12-, and 18-month recalls. Five patients did not attend the 60-month recall, because they moved to other cities (Figure 1).

Retention/fracture

After 60 months, 21 restorations were lost (12 for Adper Single Bond 2 and 9 for Ambar; Table 5). According to ADA guidelines,[29] the 60-month retention rates were 55.6% for Adper Single Bond 2 and 71% for Ambar. The risk ratio for both the groups was 0.58 (95% CI, 0.29-1.18). The 95% CI interval of the risk ratio crosses the null value of 1, meaning the groups were not different from each other (p=0.32). In addition, after the 60-month recall, 6 restorations for each group showed some small fractures (Table 5). No significant difference was detected between groups at the 60-month recall (p=1.0; Table 5).

Marginal Adaptation

According to the FDI criteria, after 60 months, 12 restorations (3 classified as "B" and 3 classified as "C" for SB, and 3 classified as "B" and 3 classified as "C" for AM) showed some marginal discrepancy (Table 5). No significant difference was detected between both the groups at the 60-month recall (p=1.0; Table 5).

Marginal Staining

The evaluated restorations showed a slight increase in the marginal staining after 60-months of clinical evaluation (3 classified as "B" and 3 classified as "C" for SB, and 3 classified as "B" and 1 classified as "C" for AM). No significant difference was found between the groups at the 60-month recall time (p=0.91; Table 5).

Recurrence of Caries

After 60 months, five restorations (2 for Adper Single Bond 2 and 3 for Ambar) showed a very small and localized demineralization around restorations that suggested recurrence of caries. However, no operative treatment was required. No difference was observed for this parameter when both adhesives were compared (p=1.0; Table 5).

Postoperative Sensitivity

Six restorations showed postoperative sensitivity in the baseline (3 for Adper Single Bond 2 and 3 for Ambar), but this occurrence was not reported in the

Table 3: *World Dental Federation (FDI) Criteria Used for Clinical Evaluation*[37,38]

	Esthetic Property	**Functional Properties**		**Biological Properties**	
	1. Staining margin	**2. Fractures and retention**	**3. Marginal adaptation**	**4. Postoperative (hyper-) sensitivity**	**5. Recurrence of caries**
1. Clinically very good (A)	1.1 No marginal staining	2.1 Restoration retained, no fractures / cracks	3.1 Harmonious outline, no gaps, no discoloration	4.1 No hypersensitivity	5.1 No secondary or primary caries
2. Clinically good (B) (after correction very good	1.2 Minor marginal staining, easily removable by polishing	2.2 Small hairline crack	3.2.1 Marginal gap (50 µm) 3.2.2 Small marginal fracture removable by polishing	4.2 Low hypersensitivity for a limited period of time	5.2 Very small and localized demineralization No operative treatment required
3.Clinically sufficient / satisfactory (C) (minor shortcomings with no adverse effects but not adjustable without damage to the tooth)	1.3 Moderate marginal staining, not esthetically unacceptable	2.3 Two or more or larger hairline cracks and/or chipping (not affecting the marginal integrity)	3.3.1 Gap < 150 µm not removable 3.3.2 Several small enamel or dentin fractures	4.3.1 Premature/ slightly more intense 4.3.2 Delayed/ weak sensitivity; no subjective complaints, no treatment needed	5.3 Larger areas of demineralization, but only preventive measures necessary (dentine not exposed)
4. Clinically unsatisfactory (D) (repair for prophylactic reasons)	1.4 Pronounced marginal staining; major intervention necessary for improvement	2.4 Chipping fractures which damage marginal quality; bulk fractures with or without partial loss (less than half of the restoration).	3.4.1 Gap > 250 µm or dentine/base exposed. 3.4.2 chip fracture damaging margins 3.4.3 Notable enamel or dentine wall fracture	4.4.1 Premature/ very intense 4.4.2 Extremely delayed/weak with subjective complaints 4.4.3 Negative Sensitivity Intervention necessary but not replacement	5. 4 Caries with cavitation (localized and accessible and can be repaired
5. Clinically poor (E) (replacement necessary)	1.5 Deep marginal staining not accessible for intervention	2.5 (Partial or complete) loss of restoration	3.5 Filling is loose, but in situ	4.5 Very intense, acute pulpitis or non vital. Endodontic treatment is necessary and restoration has to be replaced	5.5 Deep secondary caries or exposed dentine that is not accessible for repair of restoration
Acceptable or not acceptable (n, %, and reasons	Aesthetic criteria	Functional criteria		Biological criteria	

Table 4: *Characteristics of the Research Subjects and the Noncarious Cervical Lesions (NCCLs) Per Group*

Characteristics of Research Subjects	**Number of Lesions**	
Gender distribution		
Male	18	
Female	17	
Characteristics of NCCLs	**Number of Lesions**	
	SB	**AM**
Shape (degree of angle)		
<45	2	1
45-90	3	3
90-135	18	17
>135	12	14
Cervico-incisal height (mm)		
<1.5	3	3
1.5-2.5	14	17
>2.5	18	15
Degree of sclerotic dentin		
1	28	24
2	1	5
3	5	2
4	1	4
Attrition facet		
Yes	9	10
No	26	25
Enamel in cervical margin		
<25%	4	5
25%-50%	31	30
Preoperative sensitivity (spontaneous)		
Yes	16	18
No	19	
Tooth distribution		
Incisor	2	2
Canines	5	9
Premolar	25	21
Molar	3	3
Arch distribution		
Maxillary	20	24
Mandibular	15	11

Abbreviations: SB, Adper Single Bond 2 (3M Oral Care, St. Paul, MN, USA); AM, Ambar (FGM, Joinville, SC, Brazil).

following recall times. No difference was observed for this parameter when both the adhesive were compared (p=1.0; Table 5).

DISCUSSION

The simplification of technique in contemporary dental adhesives has occurred at the expense of an increasing incorporation of hydrophilic monomers.[10] According to a systematic review of clinical trials published by Peumans and others,[31] two-step etch-and-rinse adhesives perform clinically less favorably than other adhesive strategies in NCCL restorations. Two-step etch-and-rinse adhesives showed an average annual failure rate of 6.2%, which means that after 5 years of clinical evaluation, an average failure rate of 31.0% will be expected. Therefore, some manufacturers added functional monomers, such as PAC (Adper Single Bond 2) and MDP (Ambar), in an attempt to significantly improve the bonding results for simplified etch-and-rinse adhesives.

PAC is a component of several adhesive systems by 3M Oral Care available in the market, among them is Adper Single Bond (also known as Single Bond, Scotchbond 1 and Adper Scotchbond 1 in some countries)—an antecessor of Adper Single Bond 2. Initially, the rationale for the use of the PAC was to provide better moisture stability.[42] However, due to the high molecular weight of PAC, some authors indicated that PAC prevents a complete infiltration of the collagen mesh, resulting in a nonuniform adhesive–dentin interface formation.[33,34] More recently, it was observed that the carboxyl groups present in polyalkenoic acids replace the phosphate ions in hydroxyapatite, establishing ionic bonding with calcium.[18] This chemical bonding mechanism followed the same adhesion–decalcification reaction described by self-etch adhesives.[11]

Only a few years ago, Sezinando and others[18] evaluated the interaction between PAC and hydroxyapatite using high-technological spectroscopy methods. The authors showed that Adper Single Bond-containing PAC chemicals interact with hydroxyapatite, in comparison to an experimental Adper Single Bond PAC-free adhesive. It is worth mentioning, this chemical interaction depends on the abundance of PAC polar carboxyl groups, which may provide a high affinity for binding.[18] According to the manufacturer, Adper Single Bond contains from 5 wt% to 10 wt% of PAC.[17,18] This fact should be responsible for the higher immediate and long-term bond strength values of the Adper Single Bond-containing PAC when compared to the experimental Adper Single Bond PAC-free adhesive.[17,18]

Among the two-step etch-and-rinse adhesives, a systematic review of *in vitro* bond strength studies

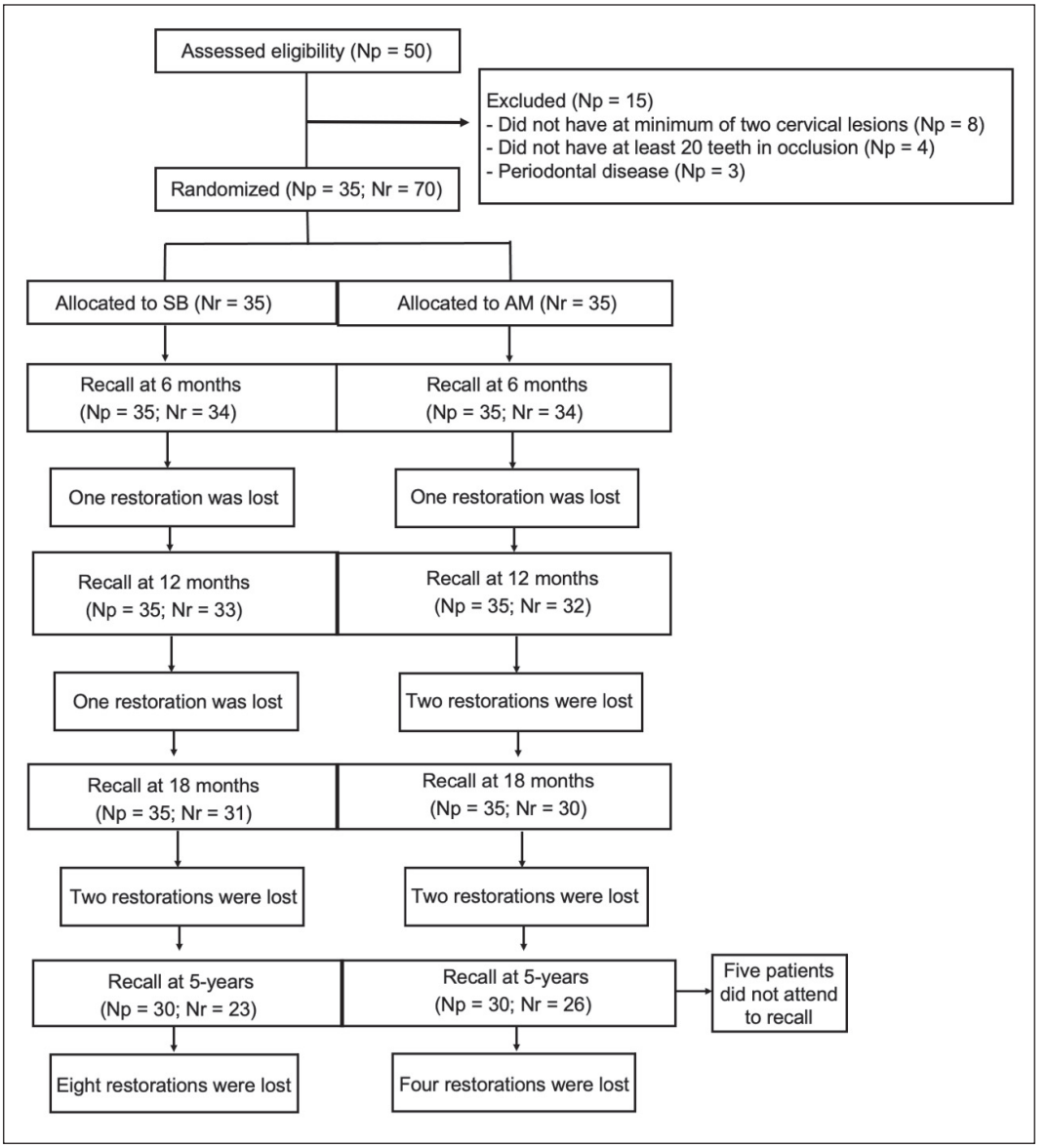

Figure 1. *Flow diagram of the study phases.*

published by De Munck and others[35] showed that Adper Single Bond (described as Scotchbond 1) had a better bond strength performance. It is worth mentioning that Adper Single Bond 2 contains nanofillers and Adper Single Bond does not. Unfortunately, the addition of nanofiller in Adper Single Bond 2 did not show improvement in terms of bonding ability.[36] However, all of these features could be responsible for the good retention rate and lower marginal discoloration of Adper Single Bond 2 in the present study, as well as the observations in the medium- and long-term clinical trial in NCCLs for their predecessor Adper Single Bond.[19-21]

Regarding the two-step etch-and-rinse adhesive, Ambar is a nanofiller- and MDP-containing adhesive. Functional monomers have already been ranked based on their chemical bonding potentials, and 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) has been identified as capable of establishing a very intensive and stable chemical interaction with hydroxyapatite. The MDP–Ca water-insoluble salts contribute to the protection of the collagen fibers. The atomic relation of the 10-MDP molecule favors the chemical interaction.[22]

Considering the chemical bonding between MDP and hydroxyapatite, dissolving the smear layer and the hydroxyapatite on the dentin surface through phosphoric acid etching, as indicated by the manufacturer of Ambar, may reduce chemical interactions mainly in the dentin surface.[37] Although

Table 5: *Number of Evaluated Restorations for Each Group Classified According to the World Dental Federation Criteria[30] in Different Follow-up Times*

Time	Baseline			6 Months		12 Months		18 Months		60 Months	
Criteria	SB[a]		AM	SB	AM	SB	AM	SB	AM	SB	AM
Fractures/Retention	VG	35	35	27	29	31	30	26	25	20	23
	GO	—	—	5	5	2	2	2	1	1	1
	SS	—	—	2	—	—		3	4	2	2
	UN	—	—	—	—	—	—		—	—	—
	PO	—	—	1	1	1	2	2	2	8	4
Marginal adaptation	VG	35	35	34	34	29	28	24	22	17	20
	GO	—	—	—	—	4	4	3	3	3	3
	SS	—	—	—	—	—	—	4	5	3	3
	UN	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—
Marginal staining	VG	35	35	34	34	33	32	26	26	17	22
	GO	—	—	—	—	—	—	4	3	3	3
	SS	—	—	—	—	—	—	1	1	3	1
	UN	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—
Recurrence of caries	VG	35	35	34	34	33	32	31	30	21	23
	GO	—	—	—	—	—	—	—	—	2	3
	SS	—	—	—	—	—	—	—	—	—	—
	UN	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—
Postoperative sensitivity	VG	32	32	34	34	33	32	31	30	23	26
	GO	3	3	—	—	—	—	—	—	—	—
	SS	—	—	—	—	—	—	—	—	—	—
	UN	—	—	—	—	—	—	—	—	—	—
	PO	—	—	—	—	—	—	—	—	—	—

Abbreviations: SB, Adper Single Bond 2 (3M Oral Care, St. Paul, MN, USA); AM, Ambar (FGM, Joinville, SC, Brazil).

[a]VG for clinically very good; GO for clinically good; SS for clinically sufficient/satisfactory; UN for clinically unsatisfactory; and PO for clinically poor.

this is the most plausible possibility, several *in vitro* studies found the resin–dentin bond strength values of MDP-containing adhesives did not diminish during water storage, even when the dentin was etched with phosphoric acid before adhesive application.[38,39] Unfortunately, there are important open questions concerning the dentin bond durability of MDP-containing adhesives when applied in the etch-and-rinse system.

Actually, a recent study published by Hidari and others[40] evaluated the effect of phosphoric acid on dentin before the application of an MDP-containing adhesive (Clearfil Universal Bond, Kuraray, Noritake Dental, Tokyo, Japan) in comparison to an MDP-free adhesive (experimental Clearfil Universal Bond). The results showed higher immediate and long-term degradation after artificial aging when a MDP-containing adhesive was used, even after phosphoric acid application. Actually, Hiraishi and others[41] speculated that a certain interaction might occur between exposed collagen fibrils and MDP. On the other hand, it is more plausible that the association of the methacrylate group with the long carbon spacer group effectively provides hydrophobicity,[42] and it might contribute to bond durability *in vivo*.[43] All of these descriptions justify the acceptable retention rate

and lower marginal discoloration for Ambar adhesive observed in the present study.

In the specific case of two-step etch-and-rinse Ambar, several *in vitro* studies showed an optimal laboratory performance, such as a higher degree of conversion inside the hybrid layer and immediate bond strength values, as well as, reduced water sorption and solubility and nanoleakage, similar to the Adper Single Bond 2.[44-48]

Actually, it is worth mentioning that an MDP-containing Ambar adhesive showed a higher retention rate (71%) in comparison to a PAC-containing Adper Single Bond 2 adhesive (55.6%). However, a closer view regarding 5-year clinical studies in NCCL when two-step etch-and-rinse adhesives were evaluated, showed that, the retention rate varied from 51.5% to 77%.[19,49-53] For instance, Van Dijken and others[50] evaluated the performance of a single two-step etch-and-rinse material, and, after 5 years, the retention rate of 62.3% was observed. In a recent paper published by Torres and others,[52] after 5 years of clinical service, a retention rate of 77% was observed when a single two-step etch-and-rinse adhesive was evaluated. Therefore, an overall analysis of clinical trials that evaluated two-step etch-and-rinse in comparison with the results of the present study does not allow us to conclude the superiority of one over the other. This clearly indicates that no significant improvement in the clinical performance of two-step etch-and-rinse adhesives were observed when PAC (Adper Single Bond 2) or MDP (Ambar adhesive) were added.

Although the two tested adhesives have several differences in their chemistry, they share important features. The Adper Single Bond 2 and Ambar adhesive system both contain ethanol as the solvent. Usually, acetone-based systems have been reported to be more sensitive to the dentin moisture than ethanol and ethanol/water adhesives.[54] If dentin is not kept sufficiently moist, the acetone-based systems cannot infiltrate within the collagen fibrils leading to reduced bond strengths.[54] This is the main reason that the majority of adhesive systems available in the market, at the present moment, are ethanol-based systems.

Although the two products differ in the kind of structural monomer employed, with Ambar containing urethane dimethacrylate (UDMA) and Adper Single Bond 2 containing the less flexible *Bis*-GMA (bisphenol A-glycidyl methacrylate),[26] this difference did not appear to produce important variances in the performance of either of the materials, at least in the evaluation period, as well as also shown in several clinical studies.[19,49-53]

Finally, although the FDI criteria was launched in 2007 for evaluating dental restorations,[30] few publications have used it.[55,56] However, at least two studies suggested that the FDI criteria is more sensitive for identifying differences in restorations than the traditional United States Public Health Service (USPHS) criteria when evaluating restorations in NCCLs. [55,56] This is the reason why the FDI criteria were used in the present study instead of the traditional USPHS criteria.

CONCLUSION

The present study demonstrated that both two-step etch-and-rinse adhesives, Adper Single Bond 2—a polyalkenoic acid-containing adhesive, and Ambar—an MDP-containing adhesive, had comparable clinical performances after 60 months of clinical evaluation.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies of The Ethics Committee on Investigations Involving Human Subjects by State University of Ponta Grossa. The approval code issued for this study is 14918/10.

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

The Effect of Radiotherapy on the Marginal Adaptation of Class II Direct Resin Composite Restorations: A Micro-computed Tomography Analysis

B Oglakci • D Burduroğlu • AH Eriş • A Mayadağlı • N Arhun

Clinical Relevance

When restorations are placed below the cemento-enamel junction after radiotherapy, a universal adhesive system with the application of the etch-and-rinse mode might be preferred.

SUMMARY

This laboratory study was designed to evaluate the marginal adaptation of Class II mesio-occluso-distal (MOD) restorations at the cervical region with micro-computed tomography (micro-CT). Two groups of restorations were compared: 1) those that had been exposed to radiotherapy before restoration was performed using a universal adhesive in etch-and-rinse and self-etch modes; and 2) those that had previously been restored using a universal adhesive in etch-and-rinse and self-etch modes and had subsequently undergone radiotherapy.

Sixty intact human molars were randomly divided into groups according to irradiation status: no radiotherapy (control group); radiotherapy followed by restoration (radiotherapy-first group); and restoration followed by radiotherapy (restoration-first group). These three groups were then subdivided into two groups each on the basis of

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adhesive application type (etch-and-rinse and self-etch modes), for a total of six groups (n=10/group). Standardized Class II MOD cavities were prepared. A universal adhesive (Clearfil Universal Bond Quick, Kuraray, Okayama, Japan) was applied. The teeth were restored with resin composite (Estelite Posterior Quick, Tokuyama, Tokyo, Japan). The radiotherapy protocol was conducted with 60 gray (Gy) at 2 Gy/day, five days a week for six weeks. Adhesive defects were analyzed in distal and mesial views and evaluated with micro-CT (SkyScan 1174v2, Kontich, Antwerp, Belgium) on the basis of the volume of black spaces between the cavity walls and the restorative materials (mm^3). The data were analyzed using the Kruskal-Wallis, Mann Whitney U and Wilcoxon tests ($p<0.05$).

The radiotherapy protocol did not affect the marginal adaptation of the universal adhesive at the cervical regions. Regarding the application modes, for the radiotherapy-first group, the self-etch mode caused significantly higher adhesive defects than the etch-and-rinse mode at the dentin margin. For the no-radiotherapy group, the adhesive defects at the dentin margin were significantly higher than at the enamel margin with the application of the etch-and-rinse mode.

INTRODUCTION

Head and neck cancer (HNC) represents 4% of cancer incidence worldwide and causes 360,000 deaths annually.[1] Malignancies in the head and neck region comprise salivary gland tumors, squamous cell carcinoma, thyroid cancer, and also hematological malignancies such as lymphoma or myeloma.[2] Radiotherapy is a mandatory component of modern cancer therapy, in combination with chemotherapy and surgical management. This treatment includes irradiation of the tumor mass with ionizing radiation. Modern radiation therapy approaches aim to preserve neighboring vital tissue function while giving the tumor a tumoricidal dose. The majority of radiation-induced biological damage originates from the reaction of the target tissue with free radicals, including hydroxyl radicals (OH) and hydrated electrons generated by the action of radiation on water. This irradiation mechanism supports the consensus of dental literature that radiotherapy of the head and neck region affects the dental tissues.[3]

Previous studies have indicated that alterations in the nature of enamel, dentin, and the dentino–enamel junction are mostly dose and mineral/organic content dependent; high doses of radiotherapy can jeopardize the stability of these structures.[4,5] The adverse effects of radiotherapy comprise the lack of the enamel prism, obliterated dentinal tubules, collagen fiber degeneration, gap formation at the enamel-dentin junction and decrease in microhardness.[6] In addition, random changes to the aromatic and aliphatic bonds of the organic matrix have been detected in irradiated composite resins.[7]

From a mechanical perspective, the aforementioned changes in dental structures have directed the attention of researchers to the potential adhesion impairment effects of irradiation. The studies have mostly concentrated on micro/macro bond strength tests with different surface conditioning protocols.[8,9,10] However, the conclusions have been contradictory, and no consensus has been reached about guidelines for the restoration of irradiated teeth with direct resin technology. The controversies about bond strength values have mostly been based on the adhesive systems used.[8,10,11,12]

The latest generation of adhesives are referred to as "universal adhesives" and have been extensively implemented due to their versatility.[13] Universal adhesives offer advantages to clinicians because of their user-friendly, simplified application protocols and multi-mode applicability to various substrates with etch-and-rinse and self-etch modes.[14] Dental literature about universal adhesive systems applied to irradiated tooth structures is scarce[8,9] and limited to bond strength testing and the examination of fracture patterns.

The cavity adaptation of a resin composite restoration predominantly determines the overall quality and longevity of the restoration, which is affected by several factors, such as substrate type (for example irradiated, eroded, or affected dental substrates), polymerization shrinkage, the type of adhesive system, and the skill of the operator. These factors impact adhesion, and microgaps may occur at the resin-dental substrate interface. Bacterial leakage and secondary caries formation basically originate at the cervical margins of Class II restorations.[15] Today, modern technologies are used to analyze marginal adaptation without destroying the samples. These include optical coherence tomography and micro-computed tomography.[16,17]

There are limited data in the literature about the marginal adaptation of universal adhesive systems at irradiated enamel and dentin substrates. Therefore, the aims of this laboratory study were to use micro-CT to evaluate the marginal adaptation of Class II MOD restorations at the cervical regions located in enamel and root dentin: 1) that have undergone radiotherapy; and 2) that have already been restored using a universal

adhesive in etch-and-rinse and self-etch modes and have subsequently undergone radiotherapy.

The research study's null hypothesis was as follows: Irradiation would not affect the marginal adaptation of Class II MOD restorations made using a universal adhesive applied in self-etch and etch-and-rinse modes at the cervical regions located in enamel and root dentin.

METHODS AND MATERIALS

The local ethics committee approved this laboratory study (Process no. 11/265).

Sample Size Calculation

The sample size was determined on the basis of the estimated effect size between groups, in accordance with the literature.[18] In the present study, 10 samples were required for each group to obtain a medium effect size (d=0.50), using 95% power and a 5% type 1 error rate.

Sample Preparation and Restorative Procedures

A total of 60 intact human molars, free of caries, were obtained, cleaned, and stored in saline solution until testing. Figure 1 provides a schematic illustration of the experimental protocol. The restorative materials, lot numbers and composition used in this study are summarized in Table 1.

The teeth were randomly divided into three main groups by one author (DB) according to exposure to and timing of irradiation (n=20), and each main group was divided into two groups according to the adhesive application type (n=10).

Standardized Class II mesio-occluso-distal (MOD) cavities (2.5 mm occlusal depth, 4 mm width, 4 mm depth at mesial box and distal box, depth 1 mm beyond the cemento-enamel junction) were prepared in each tooth with a coarse diamond fissure bur (FC Diamond, G&Z Instrumente, Lustenau, Austria). In the mesial proximal box, 4-mm depth preparations were performed in all teeth to achieve a cervical margin on the enamel surface, while in the distal proximal box, cemento-enamel junctions were visually determined and preparations performed 1 mm beyond them to obtain a cementum margin.[19] A digital caliper was used to validate the dimensions of the cavity preparation. The floor of the mesial and distal boxes was controlled for presence of enamel and cementum with a stereomicroscope (SMZ 1000, Nikon, Japan), respectively.

Group 1) Control (no-radiotherapy) group with etch-and-rinse mode—This group did not receive radiotherapy. Standardized Class II MOD cavities were prepared.

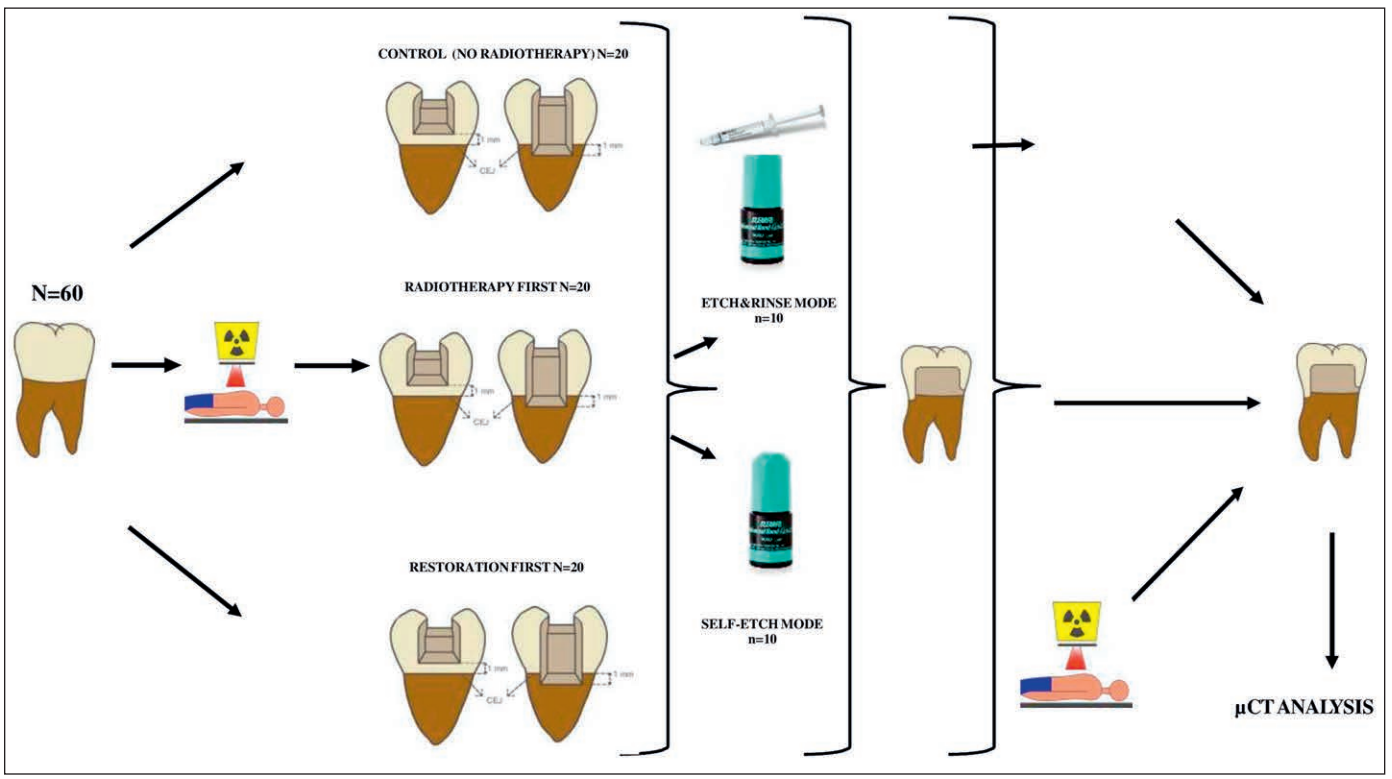

Figure 1. *A schematic illustration of the experimental protocol.*

Table 1: *The Brand Names, Lot Numbers and Composition of Restorative Materials Used in this Study*

Brand Names	Lot Number	Composition
Scotchbond Universal Etchant 3M Oral Care (St Paul, MN, USA)	6870788	32 wt% phosphoric acid, 60% water, 5% synthetic amorphous silica
Clearfil Universal Bond Quick (Kuraray, Okayama, Japan)	000036	10-MDP, Bis-GMA, HEMA, hydrophilic amide monomers, Colloidal silica, Silane coupling agent, Sodium fluoride, Camphorquinone, Ethanol, Water (pH=2.3)
Estelite Posterior Quick (A2 Shade) (Tokuyama Dental Corp, Tokyo, Japan)	W143	Organic Matrix Composition: Bis-GMA, TEGDMA, Bis-MPEPP, Radical-Amplified Photopolymerization initiator technology (RAP) Inorganic Filler Particulate: (83% wt, 70% vol) Silica-zirconia filler: 0.1-10 μm (2 μm)

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; BIS-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; Bis-MPEPP, bis-methacryloxyethoxy phenyl propane; mm, micrometer; wt%, weight percentage; vol%, volume percentage.

The enamel and dentin surfaces were etched with 37% phosphoric acid (Scotchbond Universal Etchant, 3M Oral Care, Monrovia, CA, USA) for 15 seconds, rinsed with water for 5 seconds, and blot-dried with a cotton pellet. Clearfil Universal Bond Quick (Kuraray) was applied with a rubbing motion with a microbrush for 20 seconds, air-dried until the bond did not move, and light-cured for 10 seconds (irradiance of 1000 mW/cm^2) with a light-emitting diode (LED) light-curing unit (Valo, Ultradent, South Jordan, UT, USA). The light intensity was checked with a radiometer (Demetron LED Radiometer, Kerr Corp, Orange, CA, USA). A metal auto matrix (SuperMat assorted kit, Kerr) was placed around the tooth. The resin composites were applied in 2-mm increments. For each increment, the resin composites were light-cured for 10 seconds. After removal of the auto matrix, the composite resin was light-cured again from the distal and mesial surfaces for 10 seconds on each side.

Group 2) Control (no-radiotherapy) group with self-etch mode—This group did not receive radiotherapy. Standardized Class II MOD cavities were prepared. Clearfil Universal Bond Quick was applied without acid etching. The rest of the restoration procedures were applied as described for the etch-and-rinse group.

Group 3) Radiotherapy-first group with etch-and-rinse mode—The samples first received radiotherapy in accordance with the experimental protocol for six weeks. After radiotherapy, standardized Class II MOD cavities were prepared. The adhesive system with etch-and-rinse application and restorative procedures was applied as described above.

Group 4) Radiotherapy-first group with self-etch mode—The samples first received radiotherapy in accordance with the experimental protocol for six weeks. After radiotherapy, standardized Class II MOD cavities were prepared. The adhesive system with self-etch application protocol and restorative procedures was applied.

Group 5) Restoration-first group with etch-and-rinse mode—Standardized Class II MOD cavities were prepared. The adhesive system with etch-and-rinse application protocol and restorative procedures was applied as mentioned before. Then the samples received radiotherapy in accordance with the experimental protocol for six weeks.

Group 6) Restoration-first group with self-etch mode—Standardized Class II MOD cavities were prepared. The adhesive system with self-etch application and restorative procedures was applied as mentioned before. Then the samples received radiotherapy in accordance with the experimental protocol for six weeks.

All restorations were finished and polished with an extra-fine diamond bur (FC Diamond, G&Z) and a one-step polisher (Opti1step Polisher, Kerr) according to the manufacturer's instructions. The samples were kept in distilled water at 37°C until analysis. All cavity preparations and restorative procedures were performed by a single operator who was blinded to the presence of irradiation (BO).

Radiotherapy Protocol

Irradiation of the teeth was performed at the radiotherapy center of the oncology clinic. Prior to the irradiation, the output dose of the device and deep-dose tables were used. Manual planning was performed to mimic the clinical scenario of an adult patient with HNC. During the planning process, the analytical anisotropic algorithm dose calculation was employed to provide the same radiation dose to the samples. The roots of the teeth were embedded in modeling wax contained in a plastic box. The wax surface was set at a distance of 2 mm from the cemento-enamel junction and the teeth positioned 0.5 cm apart from each other to prevent scattering and to allow for direct irradiation. Then the box was filled with distilled water in order to imitate the oral cavity.[5]

A Cobalt60 CisBio International CIRUS model teletherapy device (312TBq, Healvita GmBH, Vienna, Austria) was used. Collimators made of tungsten, steel, and natural uranium mixtures allowed the rays to fall on the treatment surface more evenly and form a homogeneous dose. The distance of the material surface from the source was detected as 80 cm, and the surface area to be irradiated was 12 x 12 cm^2. The irradiation dose rate was determined as 29.83 centigray (cGy)/min, the irradiation room temperature was 24°C, the pressure (P) was 1019 hectopascals (hPa), and the humidity was kept at 60%. Fixed irradiation was performed with a 98% deep dose. Teeth were kept 2 cm deep from the surface and 1.25 mega electron-volt (MeV) gamma rays were applied to the samples. The radiotherapy protocol was performed as 30 fractions daily, 2 Gy per fraction, five days a week for six weeks, and the total given dose was calculated as 60 Gy.[20] A dosimeter was used to control the quality of irradiation. An experienced physician performed the whole radiotherapy protocol (AHE).

Micro-CT Analysis

The analysis of marginal adaptation was done using a micro-CT device (SkyScan 1174v2, Bruker). The samples were fixed in the scanning chamber and scanned at 24.21 µm pixel size and 512 x 652 resolution for an exposure time of 2500 milliseconds. The micro-focus X-ray source was set at 50-kVp (peak kilovoltage) accelerating voltage, 800 µA (microampere) beam current and 40 W power, using a 0.25 mm Al filter. Each sample was scanned over 360 degrees with a rotation step of 0.90 degrees and with an approximately average scanning time of 40 minutes. For each sample, 400 raw data points were recorded in tagged image file format (TIFF) and reconstructed with NRecon (Ver. 1.6.10.2, Micro Photonics Inc, Allentown, PA) software; approximately 339 transverse tomographic sections were obtained in bitmap file format (BMP).

Image analyses of adhesive defects, based on the volume of black spaces, were carried out with three-dimensional analysis from CTAn software (CT-Analyser software, Version 1.16.4.1; SkyScan), and were used to create quantitative parameters and visual models and enabled densitometric and morphometric measurements. Black spaces were detected from the volumes of interest (VOI), which were created from all two-dimensional images in the region of interest (ROI) (Figure 2). A threshold value was determined in the histogram to differentiate the voxels of the sample to be examined and the voxels of the surrounding air. The threshold value was detected on the histogram where the black voxel is denoted with 0 and represents the minimum intensity, and the white voxel is denoted with 255 and indicates the maximum intensity. The volumetric rates were calculated separately with the determined ROIs and threshold value data. The data of the samples were transferred to CTVol (Ver. 2.3.2.0, SkyScan) software and three-dimensional modeling images of the samples were obtained (Figure 3). The images were recorded from the buccal to the lingual surfaces and from the outer surface to the axial wall for each proximal area per sample. In the mesial and distal views, the adhesive defects were quantified through analysis between the cavity walls, and the restorative materials were determined in mm^3.

Statistical Analysis

Statistical analysis was conducted using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was first used to indicate the normality of variables, and the data were then analyzed using the Levene test for homogeneity of variances. The data were analyzed with nonparametric tests since they did not satisfy parametric test assumptions. The Kruskal–Wallis test was performed to compare between-group differences according to the radiotherapy protocol. The Mann–Whitney-U test was used to compare between-group differences according to the application type. The Wilcoxon test was used to compare within-group differences according to the tooth substrate. Statistical significance was considered at a confidence level of 0.05 for all analyses.

RESULTS

The mean adhesive defects with standard deviations and median values (mm^3) obtained with micro-CT for all tested groups are presented in Table 2. When comparing the radiotherapy protocols, there were no significant differences in marginal adaptation

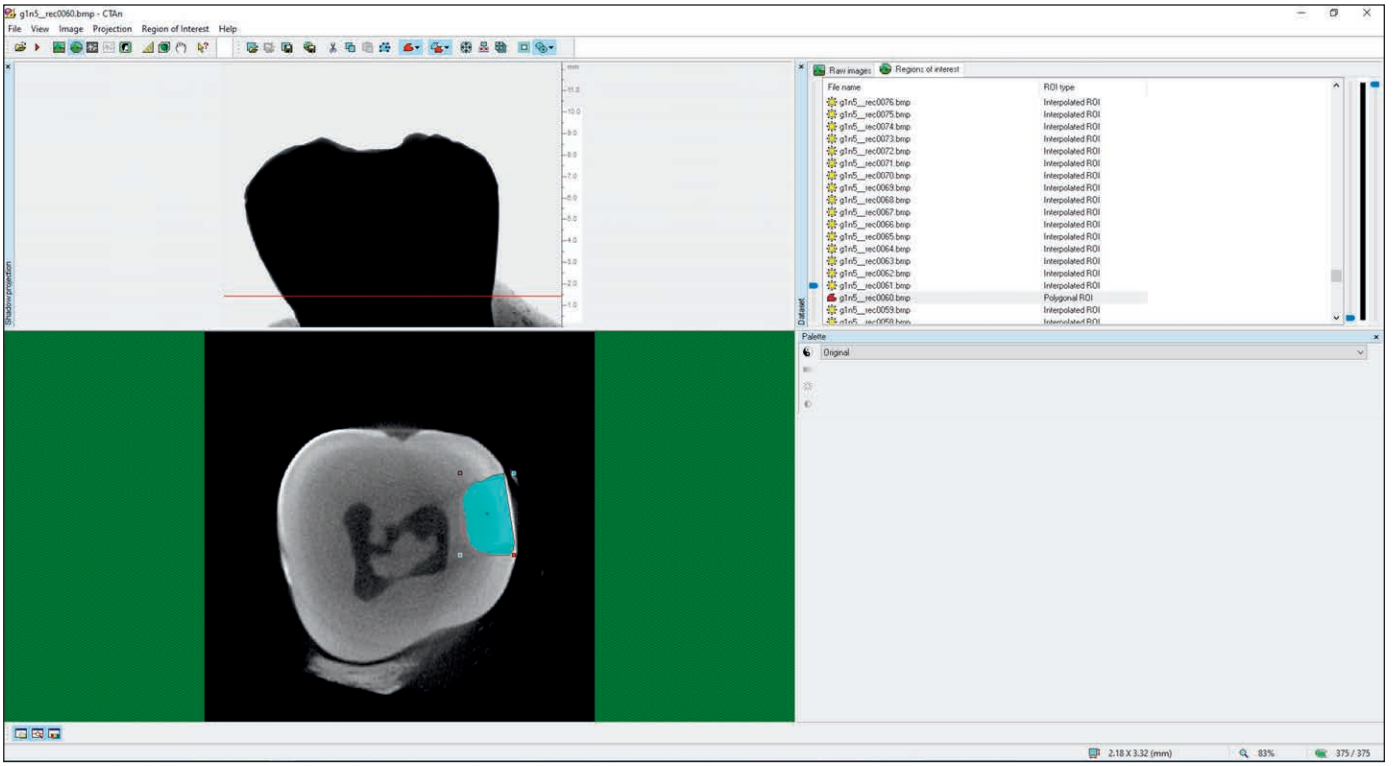

Figure 2. *Representative images of the sample analysis using micro-CT. A software version 1.16.4.1, of the volume of black spaces (adhesive defects). Two-dimensional (2D) axial sections of the restoration, showing the region of interest (ROI; blue square).*

with either the etch-and-rinse or self-etch mode applications for enamel and dentin margins ($p>0.05$). Regarding the application modes, the self-etch mode caused significantly higher adhesive defects than the etch-and-rinse mode for dentin for the radiotherapy-first group ($p<0.05$). No significant differences in marginal adaptation were detected among application modes on enamel for all tested groups with respect to the radiotherapy application types ($p>0.05$). When comparing the enamel and dentin substrates, adhesive defects for dentin were significantly higher than for enamel with the application of the etch-and-rinse mode for the no-radiotherapy group ($p<0.05$). No significant differences in marginal adaptation were observed between enamel and dentin, irrespective of the adhesive application mode and irradiation type, for the other tested groups ($p>0.05$) (Figure 4).

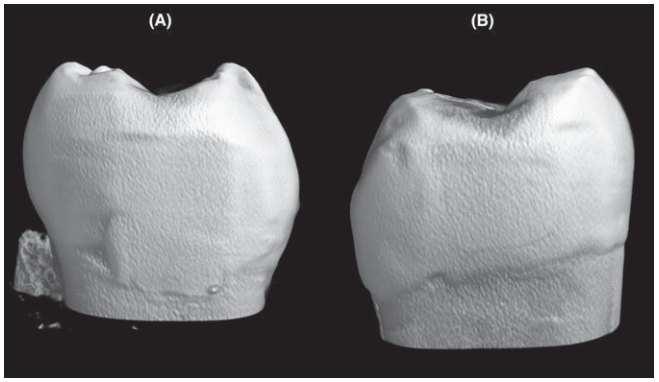

Figure 3. *Representative three-dimensional volume modeling images of the samples: distal (A) and mesial (B) views.*

DISCUSSION

In this study, the marginal adaptation of Class II MOD restorations at the cervical regions located in enamel and root dentin: 1) that had undergone radiotherapy; and 2) that were already restored using a universal adhesive in etch-and-rinse and self-etch modes and had subsequently undergone radiotherapy, were evaluated with micro-CT. Based on the results, the null hypothesis, that irradiation would not affect the marginal adaptation of Class II MOD restorations at the cervical regions located in enamel and root dentin using a universal adhesive applied with self-etch and etch-and-rinse modes, was partially rejected.

In the context of increasing dental awareness and an aging population, more dental patients are diagnosed with head and neck cancer that requires radiotherapy. Thus, clinicians should be aware of the effects of radiotherapy on dental tissues.[21] Reduced microhardness and lower stability of the dentinoenamel junction in dental hard

Table 2: *Mean Adhesive Defects with Standard Deviations (SD) and Median Values (mm³) Obtained with Micro-CT for all Tested Groups (p=0.05)*

		Below the Cemento-Enamel Junction (Dentin)			Above the Cemento-Enamel Junction (Enamel)			*p*-values Between Enamel and Dentin	
		Etch&rinse (ER)	Self-etch (SE)	*p*	Etch&rinse (ER)	Self-etch (SE)	*p*	ER	SE
No radiotherapy	Mean values ± SD	0.254 ± 0.242	0.172 ± 0.100	0.481	0.095 ± 0.053	0.087 ± 0,053	0.481	0.017	0.074
	Median values	0.175 [0.107-0.310]	0.167 [0.070-0.279]		0.084 [0.062-0.121]	0.064 [0.049-0.130]			
Radiotherapy first	Mean values ± SD	0.106 ± 0.092	0.259 ± 0.159	0.023	0.080 ± 0.055	0164 ± 0.146	0.123	0.646	0.074
	Median values	0 101 [0.015-0.186]	0.193 [0.122-0.425]		0.060 [0.042-0.110]	0.100 [0.055-0.243]			
Restoration first	Mean values ± SD	0.122 ± 0.068	0.138 ± 0.135	0.853	0.090 ± 0.071	0.131 ± 0.150	0.739	0.333	0.959
	Median values	0.131 [0.049-0.177]	0.070 [0.052-0.238]		0.072 [0.031-0.142]	0.096 [0.031-0.149]			
p		0.123	0.078		0.802	0.598			

tissues after irradiation have been reported as adverse results of radiotherapy.[22,23] It is noted that the properties of restorative materials, such as surface roughness, flexural strength, and water sorption, could be affected by radiotherapy.[24] In addition, it has been indicated that the tooth-restoration interface could be negatively influenced by degenerated collagen network, obliterated dentin tubules, and loss of enamel prism in the hybrid layer.[25] The disarrangement of the crystalline portion of the enamel and a denaturation of the organic matrix, which induces changes in the crystalline organization and protein interprismatic links, have been observed.[26] Furthermore, Cheung and others[27] have reported that irradiation might destroy the chemical bonds of restorative materials and consequently weaken their adhesion at the tooth-restoration interface. Therefore, adhesive systems have to be selected according to the substrate, and the selection of the most appropriate restorative material may be important in cases in which irradiation is involved.

The radiotherapy protocol used in this study was based on a previous study and actual clinical scenarios

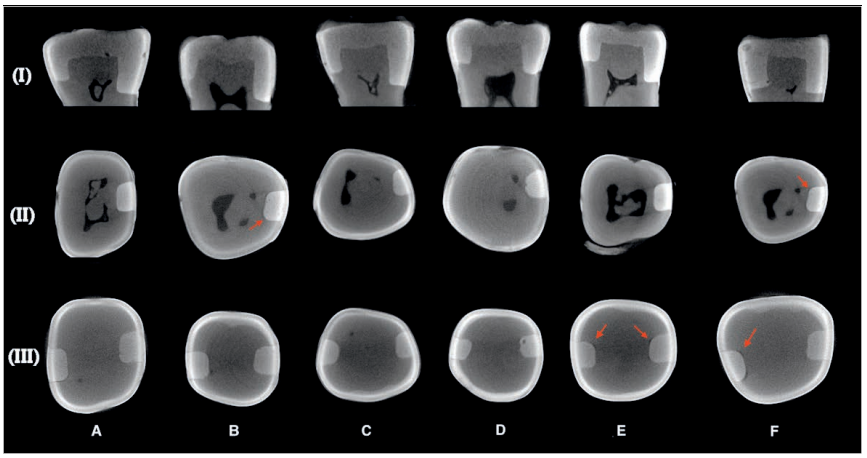

Figure 4. *Representative two-dimensional (2D) micro-CT images of all tested groups. The adhesive defects are detected between teeth and restorations (red arrows). Illustrative 2D images of the specimens are visualized: sagittal section (I), axial section for cementum margin (II) and axial section for enamel margin (III). (A): control (no-radiotherapy) group with etch-and-rinse mode. (B): control (no-radiotherapy) group with self-etch mode. (C): radiotherapy-first with etch-and-rinse mode. (D): radiotherapy-first with self-etch mode. (E): restoration-first group with etch-and-rinse mode. (F): restoration-first group with self-etch mode. Red arrows: adhesive defects.*

which contained cumulative fractioned doses of 2 Gy daily on weekdays, up to the final dose of 60 Gy.[28] Doses are generally fractioned between radiation sessions to allow time for the tumor cells to be oxygenated, making them more sensitive to irradiation; this also considers the difference in sub-lethal repair responses between tumor tissue and normal tissue.[29] The study design used molar teeth, since molar teeth have been found to receive the highest dose of irradiation during radiotherapy.[20] During radiotherapy the samples were kept in distilled water, since submersion in artificial saliva could hinder proper delivery of the irradiation because of its viscosity and high concentration of ions.[30]

Micro-CT is an imaging tool which acquires images of the three-dimensional structures of small objects with a high level of spatial resolution. Due to the penetrating capacity of X-rays, this method has been widely used to analyze the cavity adaptation of restorations without sectioning the samples and ensure the acquisition of precise information.[18,31,32] Thus, in this study, micro-CT was used to quantify the adhesive defects between the cavity walls and the restorative materials as the volume in mm^3.

Effective adhesion between the cavity walls and the restorative materials is one of the main goals in operative dentistry.[33] Previous studies have indicated that adhesive defects were mostly detected in the marginal walls and internal areas, especially on the mesiodistal and buccolingual walls of restorations.[34,35] Adhesive defects can originate from insufficient bonding at the tooth-restoration interface as a result of these factors: degradation of the adhesive layer, polymerization shrinkage, different thermal expansion coefficients between the dental substrates and the resin composite, poor application technique, and poor finishing and polishing procedures.[36] Thus, the evaluation of both internal and marginal adhesive defects was needed to evaluate in detail the properties of the restorative materials.[37]

The polymerization of composite resins creates stresses because of their contraction. These stresses could be carried to the restoration margins, possibly influencing the marginal quality.[38] When the marginal quality is inadequate, it can lead to plaque accumulation, discoloration, hypersensitivity, gap formation, bacterial leakage, recurrent caries, pulpal irritation, and consequent loss of restoration.[37,39] Thus, marginal adaptation is considered a main factor affecting the longevity of composite resin restorations.[40]

Recurrent caries is a multifactorial disease, involving marginal sealing and the characteristics of the material, the type of dental substrate on which the composite will be placed and bonded, the cavity size, the position of the tooth in the mouth, and the caries risk of the patient.[41,42] The gingival margin of Class II restorations is the most susceptible configuration for recurrent caries and also the place where maladjustments, misfits, and gaps occur, usually located gingivally (at cervical margins).[40,42] Moreover, when restorations are placed below the cemento-enamel junction, the quality of marginal integrity is doubtful.[17] In this study, cervical margins in mesial and distal views of Class II restorations were evaluated because they are the type of restorations with the highest incidence of recurrent caries formation.

It is well known that the cavity configuration (C-factor), the ratio between bonded and unbonded surfaces of the composite restoration, plays an important role in polymerization shrinkage.[36] Cavities with a high C-factor and those with large dimensions exhibit increased polymerization shrinkage and decreased bond strength. In particular, though manifesting a lower C-factor compared to Class I cavities, large Class II cavities with dentin and cementum margins[43] are susceptible to damage to marginal integrity due to polymerization shrinkage. This shrinkage stress is still a relevant trigger for the failure of restorations because of the impairment of the adhesion,[44] in particular, as the pulpal floor interface seems to be a weak spot for the effects of shrinkage stress on the adhesion of the tooth-restoration interface.[43]

A new universal adhesive system, Clearfil Universal Quick (Kuraray Noritake Co., Tokyo, Japan), containing 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) and a multifunctional hydrophilic amide monomer, has been introduced.[45] This adhesive system is used after a short time, following a "no-wait" concept: it is light-cured without waiting and features a mildly acidic pH.[46] Previous studies have reported that this monomer exhibits resistance to hydrolysis and a high bond strength.[45,47] In addition, it has good wettability to dental substrates because of the fact that amide monomer is more hydrophilic than 2-hydroxyethyl methacrylate (HEMA).[47] In particular, it can provide good adhesion in the cervical region because, due to a shorter manipulation time, the bonding procedure is not exposed to adhesion-impairing factors such as moisture in the oral cavity, gingival crevicular fluids, or bleeding from the gingiva.[48]

Ionizing radiation induces the action of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anion, and hydrogen peroxide.[30] Reactive oxygen species can be generated in dental tissues with higher water content levels, such as dentin, but also in the storage media of teeth subjected to *in vitro* radiotherapy. In addition, although water constitutes

a very small portion of enamel, its presence affects the mechanical properties of the enamel structure when it is dehydrated.[49] Irradiation significantly decreases the intrinsic resistance of enamel and dentin, with a deleterious effect on their proteic components, decreasing the stability of dentinal tissues.[50] In addition, ROS can act as a polymerization inhibitor of the adhesive system, affecting its immediate bond strength to enamel or dentin.

To the authors' knowledge, this is the first laboratory micro-CT analysis examining the quality of the marginal adaptation of direct resin restorations of irradiated teeth. Most of the literature on this issue consists of bond strength evaluations or the analysis of microleakage with visual imaging by SEM. In this study focusing on the radiotherapy protocol, no significant differences in marginal adaptations were observed with either the etch-and-rinse or the self-etch mode applications for enamel and dentin margins. Bulucu and others[12] have evaluated the effect of radiotherapy on the microleakage of enamel and dentin margins with Class V restorations using the self-etch and etch-and-rinse adhesive systems and have reported no statistically significant differences between the restoration-first and the no-radiotherapy groups, for both enamel and dentin substrates, in terms of microleakage. However, chemical alterations of dental microstructures were highlighted and the effect of the composition of the adhesive system on the achievement of successful adhesion was emphasized.[12] By comparison, Jornet and others,[26] evaluating the effect of daily applications of artificial saliva, fluoride mouth rinses, and chlorhexidine on microleakage in Class V irradiated bovine teeth, reported that a significant increase in microleakage was detected for composite resin restorations after radiotherapy. Furthermore, in the present study, the self-etch application mode caused significantly higher adhesive defects than the etch-and-rinse mode on dentin for the radiotherapy-first group. However, the application modes did not significantly affect the marginal adaptation for other tested groups. This finding could be explained by the fact that the shorter application time of this adhesive system in the self-etch mode might have resulted in insufficient removal of the smear layer and infiltration of the resin monomers to obliterated dentinal tubules. Bulucu and others[12] have indicated that etch-and-rinse adhesive systems caused significantly higher microleakage than self-etch adhesive systems on dentin for the restoration-first and no-radiotherapy groups.

Enamel contains organic components and some water, although significantly less than dentin. Therefore, it is not exclusively an inorganic tissue.[12] It has been reported that adhesion to enamel resulted in less adhesive defects and greater stability than bonding to dentin due to dentin's tubular structure and intrinsic wetness.[51] Thus, effective and durable adhesive systems are needed to obtain better cavity adaptation. In particular, the absence of enamel at the cervical margin could lead to weak adhesion of restorations. Cheung and others[27] have indicated that irradiation damage of collagen fibers could lead to decreased bond strength between dentin and composites. In this study, adhesive defects with dentin were significantly higher than with enamel with the application of the etch-and-rinse mode for the no-radiotherapy group. Previous studies[14,52] have reported that bond strength to dentin decreased with the phosphoric acid etching of dentin before the application of the adhesive system. The main reason for this decrease in bond strength has been reported to be the incomplete resin monomer infiltration of the deeply demineralized collagen network because phosphoric acid can decalcify dentin more deeply than an adhesive is designed to infiltrate.[51] In addition, in this study, no significant differences in marginal adaptation were detected between enamel and dentin for the restoration-first and the radiotherapy-first groups. This finding is in contrast with Bulucu and others,[12] who indicated that dentin had higher microleakage than enamel for the restoration-first groups. The divergence in outcomes could be attributed to the differences in adhesive systems used (etch-and-rinse and self-etch adhesives) or the aging procedure.

Regarding the limitations of the current study, the paper evaluated only the short-term effects of radiotherapy on the marginal adaptation of universal adhesive systems at cervical regions with etch-and-rinse and self-etch modes. The aging procedures of resin composite restorations are known to negatively influence cavity adaptation.[53] Furthermore, it is well known that residual reactive oxygen radicals can be responsible for unfavorable effects on the dental substrates, even when irradiation has been completed. Therefore, further studies should focus on the effect of high doses of radiotherapy on the long-term structural changes of restorations with selective etch, self-etch, and etch-and-rinse application modes.

CONCLUSIONS

The results of this study suggest that the etch-and-rinse mode of application might be preferred when the universal adhesive system is used for restorations placed below the cemento-enamel junction after radiotherapy. Within the limitations of this study, it can be concluded that:

1. The radiotherapy protocol did not affect the marginal adaptation of the universal adhesive at the cervical regions.
2. When comparing the application modes, for the radiotherapy-first group, the self-etch mode caused significantly higher adhesive defects than the etch-and-rinse mode at the dentin margin.
3. When comparing the dental substrates, for the no-radiotherapy group, adhesive defects at the dentin margin were significantly higher than at the enamel margin with the etch-and-rinse application mode.

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Regulatory Statement

The local ethics committee approved this laboratory study (Process no. 11/265).

Conflict of Interest

The authors do not have any financial interest in the companies whose materials are included in this article.

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Effect of Argon Plasma Surface Treatment on Repair of Resin Composite Aged Two Years

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Clinical Relevance

After one year of water-storage, the composite repair technique was improved with the combination of non-thermal plasma surface treatment with silanization and hydrophobic adhesive application on a sandblasted resin composite aged two years.

SUMMARY

Objectives: To evaluate the effect of argon plasma treatment (PLA) when combined with sandblasting (SAN), silanization (SIL), and hydrophobic bonding resin (HBR) on the shear bond strength (SBS) of a two-year water-aged resin composite bonded to a newly placed composite after 24 hours and one year of water-storage.

Methods and Materials: Thirty-six light-cured composite plates (20mm x 20mm x 4mm thick) were obtained and stored at 37°C in distilled water for 2 years. These aged plates were distributed into 6 groups (n=6) according to the surface treatment: no treatment (Negative Control); SAN+SIL+HBR (Positive Control); SAN+PLA+SIL+HBR; PLA+SIL+HBR; PLA+SIL; PLA+HBR. Fresh resin composite cylinders were built up using silicone molds (hole: 1.5 mm high x 1.5 mm diameter) positioned over the aged plates. Half of the SBS samples were stored in distilled water for 24 hours and loaded until failure, while the other half were stored for 1 year before being tested. Data were submitted to two-way analysis of variance and post-hoc Tukey Test (preset alpha of 0.05).

Results: Positive Control, SAN+PLA+SIL+HBR and PLA+SIL+HBR groups presented higher SBS means at the 24 hour evaluation. After 1 year of

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water storage, all groups demonstrated significant SBS reduction, with the SAN+PLA+SIL+HBR group presenting the highest SBS.

Conclusions: Resin plasma treatment in combination with other surface treatments can improve the SBS of composite repairs after one year of water storage. The SBS of the composite repair was not stable over time regardless of the surface treatment.

INTRODUCTION

The complete removal of an old composite restoration is frequently not necessary or desirable. Techniques such as simple composite repair preserve tooth structure, reduce the cost of clinical procedures and prevent the potentially harmful effects of tooth preparation on the pulp.[1,2] Studies have shown that the behavior and success of adhesion of the repaired area depend on the chemical composition of the composite restoration,[3] its wettability and roughness,[4,5] and the surface treatment protocol applied.[3,6,7,8,9]

It has been demonstrated that the bond strength between old and new composites decreases after prolonged water storage.[10] In addition, for older composite restorations, there are fewer chemical bonds available for bonding with a fresh composite, since unreacted monomers have leached out over time. To overcome this undesirable situation, surface treatments are recommended to increase the free surface energy of the composite restoration to be repaired. Sandblasting with aluminum oxide has been indicated to increase superficial roughness and create micromechanical retention at the composite surface.[11,12,13,14] In addition, a chemical bond may be established between resin and silica glass filler particles by application of a silane-coupling agent.[15,16,17,18,19]

An alternative method for treating the composite resin surface is the application of atmospheric pressure argon plasma (PLA), which increases the surface energy by surface chemical destabilization, making it possible for the composite resin surface to form new chemical bonds to another substrate.[20] Thus, PLA application to old composites may have the ability to enhance the composite restoration repair technique, improving its bond strength and longevity.[14,21,22] Several studies have evaluated composite repair bond strength using composite samples aged for a short time; however, none tested the effect of these treatments in repair techniques using two-year-aged composites.

The aim of this study was to evaluate the effect of PLA combined with sandblasting (SAN), silanization (SIL), and hydrophobic bonding resin (HBR) on the shear bond strength of a two-year water-aged restorative resin composite to a newly placed composite after 24 hours and one year of distilled water-storage. The hypotheses tested were that: 1) there is no difference among the surface treatments of old composites in their effects on repair bond strength; and 2) shear bond strength values are stable following water-storage for one year.

METHODS AND MATERIALS

Thirty-six standardized composite plates (20 mm x 20 mm x 4 mm thick) were obtained in the same way as in our previous study,[14] by placing composite resin (Charisma, Heraeus Kulzer, Hanau, Hesse, Germany, shade A2, lot number: 010611) in silicone molds. The composite surface was divided into four areas of 100 mm^2, which were light activated separately for 20 seconds; each area was fully covered by the light irradiation. The same light curing was repeated on the bottom surface. The light-curing unit used was a multiwavelength LED (Valo Cordless, Ultradent Products Inc, South Jordan, UT, USA) with 9.4-mm internal tip diameter and delivering 1,470 mW/cm^2 of irradiance (USB 4000, Ocean Optics, Dunedin, FL, USA) in standard mode. Composite plates were stored in distilled water at 37°C for 2 years. After aging, plates were polished with 600-grit SiC paper for 20 seconds (Norton, Vinhedo, SP, Brazil) to remove the outer surface and were submitted to ultrasonic cleaning for 5 minutes (USC 1400, Unique Industrio e Comercio de Produtos Eletronicos Ltda, Indaiatuba, SP, Brazil). Afterwards, plates were distributed into 6 groups (n=6) according to the following surface treatments:

1. Negative Control: old composite + new composite
2. Positive Control: old composite + SAN +SIL + HBR + new composite
3. SAN+PLA+SIL+HBR: old composite + SAN + PLA + SIL + HBR + new composite
4. PLA+SIL+HBR: old composite + PLA + SIL + HBR + new composite
5. PLA+SIL: old composite + PLA + SIL + new composite
6. PLA+HBR: old composite + PLA + HBR + new composite

The PLA (Surface Plasma Tool Model SAP - Lab applications; Surface - Engineering and Plasma Solution LTDA, Campinas, SP, Brazil) application time was 30 seconds,[14,23] using only argon gas (Praxair 4.8, White Martins Gases Ind. S.A., Rio de Janeiro, RJ, Brazil), with an output of 1.0 liter per minute.[24] Sandblasting (SAN) with 50-μm aluminum oxide particles (Microetcher, Danville Materials, San Ramon, CA, USA) was performed for 10 seconds, 10

mm distant from the plate surface at 60 psi, followed by ultrasonic cleaning (5 minutes) and air-drying for 30 seconds.

For SIL application, a drop of a silane-coupling agent (Ceramic Primer, 3M Oral Care, St Paul, MN, USA, lot number: N555194) was deposited on a mixing pad and collected by a disposable brush to be applied over the plates in a uniform thin layer. The layer was kept undisturbed for 15 minutes and air dried for 10 seconds for evaporation of water and other solvents. The HBR (Adhesive/Adper Scotchbond Multi-Purpose, 3M Oral Care, lot number: N515442) was applied in a uniform coating using a disposable brush, followed by 10 second light-activation with the same light-curing unit.

The silicone molds (Aquasil Ultra Putty, Dentsply Caulk, Milford, DE, USA) were positioned over the treated plates and a fresh composite (same brand and manufacturer - lot number: 010636A) was inserted into the mold. The fresh composite was light cured for 20 seconds and the silicon mold was carefully removed to expose a cured composite cylinder of fresh resin bonded to the aged composite plate (1.5 mm high x 1.5 mm diameter). Four composite cylinders were placed on each composite plate at four different locations on the plate, and the samples were immersed in distilled water at 37°C. Two composite cylinders on each plate were tested after 24 hours of water-storage, while the two remaining cylinders were tested after one year of water storage.

For the bond strength test, each plate was positioed on a device attached to a universal testing machine (EZ Test, Shimadzu Corp., Kyoto, Japan). A thin orthodontic wire (0.2 mm diameter) was looped around the cylinder, making contact with half of its circumference, and subjected to a shear force (crosshead speed of 0.5 mm/min). at the old-new composite bonding interface until failure occurred. Bond strength data were calculated using the peak of loading failure divided by the specimen surface area, and means were obtained in megapascals (MPa). The average value obtained from the two analyzed cylinders for each storage period was considered the mean value of each sample. Normality of the data was reached after transformation and subjected to two-way analysis of variance (ANOVA) and Tukey post hoc test ($p<0.05$), using SAS 9.3 software (SAS Institute, Cary, NC, USA).

After bond strength testing, the fracture surfaces were mounted onto brass stubs and gold coated (MED 010, Balzers, Balzer, Liechtenstein). Tested specimens were examined using a scanning electron microscope ([SEM] JSM-5600LV, JEOL Inc., Tokyo, Japan) at 35× magnification (voltage: 15 kV; beam width: 25-30 nm; working distance: 10-20 mm). The failure modes were classified as: 1- adhesive failure (at the old-new composite interface) or 2- cohesive failure within old composite.

RESULTS

Statistical testing indicated that both treatment ($p<0.001$) and evaluation-time factors ($p<0.001$) significantly influenced bond strength, with significant interaction between them ($p=0.003$) (Table 1). At 24 hours, the groups Positive Control (28.3 MPa), SAN+PLA+SIL+HBR (34.2 MPa), and PLA+SIL+HBR (28.2 MPa) presented the highest bond strength values, while Negative Control (8.8 MPa) and PLA+SIL (11.8 MPa) showed the lowest ones. Group PLA+HBR (18.4 MPa) presented an intermediate bond strength result. After one year, bond strength of all surface treatments reduced significantly and SAN+PLA+SIL+HBR (23.8 MPa) presented the highest mean, followed by Positive Control (17.6 MPa) and PLA+SIL+HBR (16.4 MPa). The other groups showed bond strength lower than 8 MPa.

Table 2 shows the results of failure modes, and Figures 1 and 2 are representative images of adhesive and cohesive failures, respectively. At 24 hours, groups Positive Control, SAN+PLA+SIL+HBR, and PLA+SIL+HBR had 100% cohesive failures, while Negative Control and PLA+SIL had 100% adhesive failures at the old-new composite interface. Group PLA+HBR presented 50% adhesive and 50% cohesive failures. At one year, an increase in the percentage of adhesive failures was observed for Positive Control (from 0% to 30%), SAN+PLA+SIL+HBR (from 0% to 20%), PLA+SIL+HBR (from 0% to 40%), and PLA+HBR (from 50% to 100%). Negative Control and PLA+SIL continued to have 100% adhesive failure.

Table 1: *Bond Strength Means (SD) for Experimental Groups (in MPa)*[a]

Group/Treatment	24 Hours	1 Year
Negative Control	8.8 (1.9) Ca	5.2 (0.8) Cb
Positive Control (SAN+SIL+HBR)	28.3 (1.8) Aa	17.6 (2.2) Bb
SAN+PLA+SIL +HBR	34.2 (2.8) Aa	23.8 (4.7) Ab
PLA+SIL+HBR	28.2 (3.0) Aa	16.4 (5.1) Bb
PLA+SIL	11.8 (1.7) Ca	2.7 (0.6) Db
PLA+HBR	18.4 (4.2) Ba	7.5 (1.5) Cb

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

[a] *Uppercase letters compare treatments within the same evaluation time and lowercase letters compare evaluation times within the same treatment (p<0.05, by Tukey test).*

Table 2: *Failure Modes (%) Among Experimental Groups*

Group/Treatment	24 Hours	1 Year
	AD/CO	AD/CO
Negative Control	100/0	100/0
Positive Control (SAN+SIL+HBR)	0/100	30/70
SAN+PLA+SIL +HBR	0/100	20/80
PLA+SIL+HBR	0/100	40/60
PLA+SIL	100/0	100/0
PLA+HBR	50/50	100/0

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

DISCUSSION

The first hypothesis—that the difference among the surface treatments of old composites would have no effect on repair bond strength—was rejected, because some groups (Negative Control, PLA+SIL and PLA+HBR) showed lower bond strength at both evaluation times than that obtained for Positive Control and SAN+PLA+SIL+HBR. The second hypothesis was also rejected, since the repair bond strength of all groups was reduced after one year, with reductions ranging from 30% (SAN+PLA+SIL+HBR) to 77.1% (PLA+SIL).

The same SAN+PLA+SIL+HBR group that showed the lowest percentage of bond strength reduction also resulted in the highest composite repair bond strength at one year (23.8 MPa). This group differed from Positive Control due to the PLA application after SAN. At 24 hours, the bond strength of these groups (Positive Control and SAN+PLA+SIL+HBR) did not show a statistical difference, but the higher percentage reduction seen with the Positive Control group (37.8%) after one year resulted in a lower bond strength mean compared with SAN+PLA+SIL+HBR. Thus, PLA positively influenced the results and showed an important role in the long-term evaluation of repair bond strength. SAN+ SIL+HBR was used as the Positive Control because a bibliographical survey showed that this technique obtained the best bond strength repair results.[11-13,19,25,26]

The correct method for the aging of composites is crucial for the analysis of their repair potential in a laboratory set-up that resembles the clinical environment. In spite of the fact that there is no aging protocol considered the gold standard for simulating the aging process that composites are subjected to in the oral environment, static storage in distilled water is the most used method for aging such composites (82.4%).[27] Besides the method, the time of aging is of utmost importance since, in clinical situations, composite repair can be expected to be necessary in the medium or long term.[28] However, only 22.2% of the studies in the current literature aged composites in water for 30 days or longer.[27] In this study, composite samples were aged by immersion in distilled water for 2 years before they were repaired and tested.

The storage of samples in composite repair studies is of great interest because new composites are more reactive than older ones. Free radicals and free monomers are still available in fresh restorations, improving their adhesive capability.[29] In contrast, over time hygroscopic

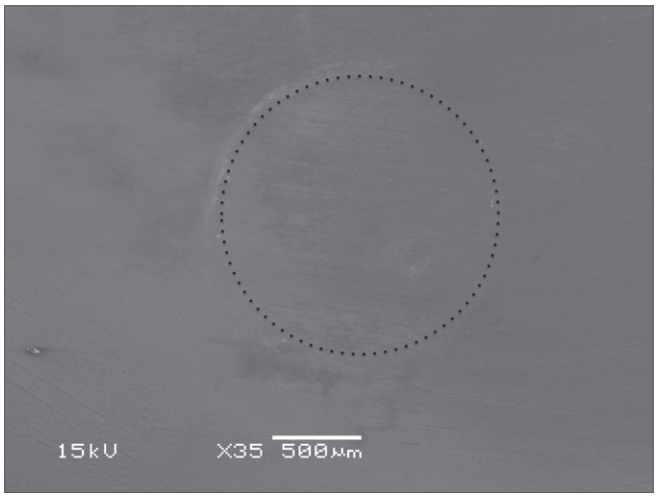

Figure 1. *Representative SEM image of adhesive failure (at the old-new composite interface). This failure occurred in a sample (at 24 hours) of the Group 5 (PLA+SIL). Original magnification 35×.*

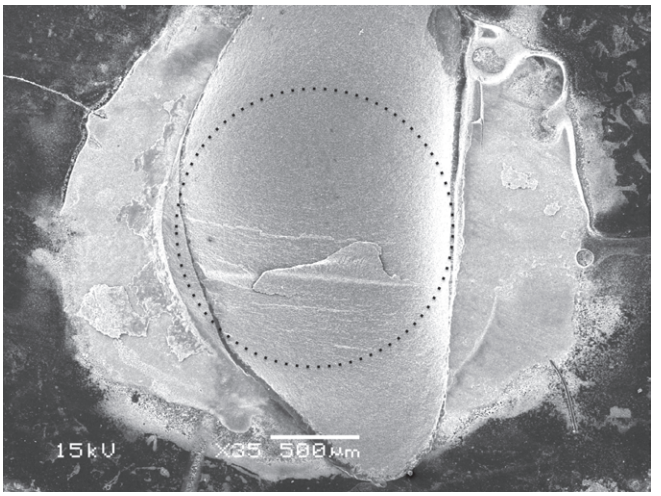

Figure 2. *Representative SEM image of cohesive failure within old composite. This failure occurred in a sample (at one year) of the positive control (SAN+SIL+HBR). Original magnification 35×.*

and hydrolytic effects of water aging take place within the cross-linked polymer structure, leading to water absorption and polymer swelling, which relax the physical bonds between polymer chains and allow free monomers and oligomers to be eluted.[27,30] Depending on the composition of old composite, the aging of a restoration can affect the materials differently. Thus, knowing the type of composite (such as microhybrid, nanohybrid, or nanofilled) to be repaired is important for the success of the procedure.[3,18,31] For example, the effects of PLA on various types of composites is not known, and further studies are needed to evaluate whether PLA can alter composite surface properties differently.

If a composite sample is aged, it has a low surface energy with poor wettability, and a surface treatment is required to improve these properties.[20] In this laboraotory study, the outer surface of old resin composite was removed using 600-grit SiC abrasive paper to flatten the surface and prepare it for bonding with fresh composite; in clinical practice, this is accomplished with carbide and diamond bur rotatory instruments. The exposed inner surface might have more residual monomers and pendant methacrylate groups for bonding to newly placed composite, potentially resulting in higher bond strength values.

PLA application is an option for increasing surface energy and making the polymeric structure more reactive to chemical bonding. PLA is a partially ionized gas consisting of a mixture of atomic and molecular reactive species.[14,20,32,33,34] In a high frequency electrical field, the free electrons become energized and collide with neutral gas molecules, and the energy transferred in the process dissociates molecules, forming numerous reactive species. The interaction of these excited species with solid surfaces increases their hydrophilicity and reactivity.[20] Argon gas is non-toxic and inert and is consumed during plasma generation. However, despite the fact that certain nonthermal plasma devices have already been approved as safe in some clinical trials, there are still many open issues with regard to the molecular and biophysical mechanisms of the procedure and the biological effects of plasma on mammalian cells and tissues.[35]

The application of argon plasma for 30 seconds has been used to treat dentin for bonding of direct composites,[36] for improving resin cement bonding to indirect composite materials[23] and zirconia ceramics,[37] and has been suggested for treating old composite restorations in repair techniques.[14] The PLA application time for 30 seconds is clinically adequate and produces the desired effect, as this is an additional step in the composite repair technique and because application times longer than 1 minute can be considered clinically long, no added benefits, and times less than 30 seconds do not have the positive effect to increase bond strength.

PLA effects may explain some outcomes of this study, such as that the group SAN+PLA+SIL+HBR presented the highest composite repair bond strength mean at one year. The ceramic primer (SIL) is a very fluid material and may not require PLA to increase wetting of the sandblasted, hydrophobic old composite, as can be seen when comparing the Positive Control and SAN+PLA+SIL+HBR groups at 24 hours. However, at one year, a statistical difference was observed between the Positive Control and the SAN+PLA+SIL+HBR group, which may be because the application of PLA results in reactive species being deposited on the composite surface, improving SIL chemical bonding to the fillers and HBR to the resin matrix of old composite. In addition, at both 24 hours and one year, the PLA+SIL+HBR group did not differ from the Positive Control (SAN+SIL+HBR), showing the potential of PLA to activate the surface and partially substitute for the SAN technique. These outcomes are different from those previously found, in which PLA did not improve bond strength when used alone or in combination with other surface treatments.[14] The same type of composite was used in the present study and in the article by Ayres and others [14]; however, the low aging time (six months) yielded amore reactive composite surface containing considerably more unreacted methacrylate groups,[38] which may have influenced the bond strength more significantly than the application of PLA. On the other hand, by aging the composite for two years, it was possible to verify the real effects of PLA application on improving the composite repair bond strength once unreacted monomers were leached out.

The air abrasion promoted by SAN is one type of roughening procedure that can provide higher composite repair bond strength than other mechanical methods. SAN produces three-dimensional roughness with variations in the peaks and valleys of the surface and thus provides more micro-retentive features than other mechanical treatments and more available area to interact with a bonding agent and the new composite increment.[11,12,14,25,26,39] Mechanical shocking by alumina particles and the non-selective removal of filler particles and portions of the polymer matrix promote retention by interpenetration of fluid material, forming a bonded interface after curing.[27]

The SAN technique contributes to the exposure of silica from the interior of the aged composite and also increases the surface area for adhesion. Old composite consists of inorganic filler particles that should be silanized to improve its bonding to organic monomers in the repair material.[19,40] One important

property of PLA application that may contribute to bond strength is its ability to change surface energy and enhance wetting of different surfaces,[24,36] possibly resulting in the resilanization of the filler particles at the sandblasted surface. This might explain why the PLA+SIL association (SAN+PLA+SIL+HBR group) demonstrated higher bond strength than SIL alone (Positive Control) in sandblasted composite after 1 year of water storage.

The groups that used SAN as surface treatment, along with the group PLA+SIL+HBR, showed higher means at 24 hours and one year, indicating the importance of SAN for immediate and long-term mechanical retention. In addition, the Negative Control and PLA+SIL groups, which did not use SAN, showed the lowest bond strength means at the 24-hour and one-year evaluation times. However, to safeguard the health and safety of patients and professionals during intraoral SAN, an aspirator device and rubber dam isolation must be used, thus limiting its clinical applicability.[41] Alternatively, to avoid SAN, rotary instruments with diamond burs may also be able to roughen restoration surfaces.[13,25,39]

Studies have suggested the use of a low-viscosity material for application after surface treatments, particularly SAN, because packable, high-viscosity composites do not penetrate into the micro-irregularities caused by air abrasion.[38,42,43] Thus, a bonding agent can improve composite repair bond strength;[44-46] this is attributed to the bonding agent's infiltration into and retention in the mechanical roughening created by air abrasion.[25] In this study, the Negative Control and PLA+SIL groups, which were not submitted to SAN and did not receive HBR, presented the lowest bond strength means at both evaluation times. Alternatively, some studies have used adhesives containing hydrophilic monomers instead of HBR.[47,48] However, highly hydrophilic bonding agents can result in the increase of water sorption and solubility on the adhesive layer, leading to early hydrolytic degradation and eventually to interfacial debonding.[25,47,48]

Despite the importance of the HBR layer, a previous study showed that an intermediate bonding agent did not improve the composite's immediate repair bond strength without SIL, which can improve repair bonding, and results in cohesive failures within composite structure after testing.[3] This fact could be seen in this study, since the PLA+HBR group did not show high bond strength means, especially after one year. Aged composites have low levels of unreacted monomers on their surfaces, and new reactions aiming to create a chemical bonding mechanism with filler particles are important for composite repair techniques. Silanes contain a silanol group (or alkoxy group), which can chemically react with the methacrylate group that copolymerizes with the resin matrix and with the hydroxyl groups from silica-based filler particles of the old composite.[49,50]

In this study, SIL combined with HBR and SAN yielded higher composite repair bond strength at 24 hours and one year. However, when used solely with PLA (PLA+SIL group) the bond strength was very low because silane chemical coupling with the old composite depends upon the availability of silica at the surface.[27] The two methods, mechanical (SAN) and physical (PLA), complement each other, and, according to a recent systematic review, the application of both physical and chemical surface treatments on aged dental composites improves the repair bond strength of methacrylate-based restorations.[27] In addition to the analysis of the influence of different types of surface treatments, the time-factor evaluation, with long-term water storage for one year, indicated that composite repair bond strength decreases over time regardless of the surface treatment.

The failure mode results in this study were in agreement with the bond-strength outcomes, since groups that presented the highest bond strength means had a low rate of adhesive failures and a high rate of cohesive failure within aged composite, showing better composite-to-composite bond strength repair quality. However, it is important to consider that the shear bond-strength test favors the occurrence of cohesive failures, mainly when there is a strong interaction between bonded materials.[51,52] The microtensile bond strength test is the preferred method of evaluating the bond strength of adhesive resins to enamel and dentin, because the stress concentration at the bonded interface is more severe in shear as compared to tension; the "macro" bond strength tests increase the rate of cohesive failure.[53] Moreover, among shear methods, the chisel as a loading device causes the most severe stress concentration at the tested interface,[53] while the orthodontic-looped wire can yield the highest shear bond strength values followed by chisel and stainless steel tape.[54] However, in studies of resin cement adhesion to ceramics and indirect resins, as well as in composite resin repair investigations, the shear strength test is still used.[3,5,7,10,13,14]

Groups that did not undergo any kind of mechanical surface treatment (Negative Control and PLA+SIL) showed 100% adhesive failure, while PLA+HBR showed an intermediate result, with 50% adhesive failure and 50% cohesive failure. The composite repair long-term bond-strength reduction was consistent with failure pattern results, since water-storage for one

year increased the number of adhesive failures for the Positive Control (30%), SAN+PLA+SIL+HBR (20%), and PLA+SIL+HBR (40%) groups.

CONCLUSIONS

Within the limitations of the shear bond strength method, PLA used in combination with other surface treatments (SAN, SIL and HBR) can improve the bond strength of composite repair after one year of water storage. When SAN was substituted by PLA, no difference was observed among the bond strength means of these groups, regardless of the evaluation time. The composite repair bond strength was not stable over time for any of the treatments evaluated. However, the best outcome was found when mechanical and chemical treatments were combined.

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Conflict of Interest

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

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Effects of Adjacent Tooth Type and Occlusal Fatigue on Proximal Contact Force of Posterior Bulk Fill and Incremental Resin Composite Restoration

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Clinical Relevance

Proximal contact effectiveness tends to decrease with occlusal fatigue loading. This effect was not detected visually using digital radiography. The bulk fill and incremental filling techniques have similar proximal contact forces.

SUMMARY

Objectives: To measure the proximal contact force in newtons (N) between incremental and bulk fill class II resin composite restorations and implant molar teeth or adjacent premolar teeth with simulated periodontal ligament.

Methods: The model used was created with a typodont first molar tooth with two bilateral occlusal-proximal class II cavities, an adjacent tooth simulating an implanted molar tooth (Titamax CM, Neodent, Curtiba, PR, Brazil) and a premolar with simulated periodontal ligament. Two resin composite restorative techniques were

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used: Inc-Z350XT, (Filtek Z350, 3M Oral Care, St. Paul, MN, USA) inserted incrementally and Bulk-OPUS, (Opus Bulk Fill APS, FGM, Joinville, SC, Brazil) high viscosity bulk fill resin composite (n=10). As a control, a typodont having intact teeth without restorations was used. After the restorative procedure, each specimen was radiographed using a digital system (Dürr Dental, Bietigheim-Bissingen, Germany). The proximal contact force (N) was measured using dental floss with a microtensile machine (Microtensile ODEME, Luzerna, SC, Brazil). The specimens were then subjected to mechanical fatigue cycling to simulate 5 years of aging. All the parameters were measured after aging. The X-rays were blindly qualitatively analyzed by two operators to identify the loss of proximal contact. One-way ANOVA was used for comparing the initial contact force between restored and intact teeth. Two-way ANOVA followed by Tukey testing was performed for contact area data and for the contact force/contact area ratio. The proximal contact force data were analyzed using one-way repeated measurement ANOVA followed by Tukey testing (α=0.05). The X-ray proximal contact analyses were described by the frequency.

Results: The initial proximal contact force was similar for intact and restored teeth. The contact force and contact area with the molar were significantly higher than with the premolar; however the contact force/contact area ratio was similar for all tested groups. The bulk fill technique showed a contact force similar to the incremental filling technique. Fatigue resulted in a significant reduction in the proximal contact force (p<0.001), irrespective of the region analyzed or restorative material used. The digital X-rays detected no alteration in the proximal contact after occlusal fatigue.

Conclusions: Larger contact area resulted in higher proximal contact force. Proximal contact force decreased with 5 years of simulated occlusal fatigue. The bulk fill technique showed a proximal contact force similar to that of the incremental filling technique.

INTRODUCTION

Resin composites have been successfully used for restoring posterior teeth, showing acceptable longevity in clinical studies.[1] More than 500 million direct dental restorations are carried out each year around the world, including approximately 261 million direct resin composite restorations.[1,2] Characteristics such as secondary caries, fracture resistance, retention, marginal adaptation and discoloration, and proximal contact of posterior restorations are the most important factors determining the clinical success of posterior restorations.[3,4]

Proximal contact occurs when the tooth or restoration contour on the distal or mesial surface remains in close contact with the adjacent tooth.[5] An adequate posterior proximal contact between adjacent teeth is related to the clinical success of restorations due to the maintenance and stabilization of the dental positions in the arches.[6] An adequate proximal contact point means that there is a space for the passage of dental floss between adjacent teeth with little resistance. The dental floss does not pass without resistance but also is not so tight as to prevent the passage of the floss or to tear it.[7] A contact point that is slightly open may cause food accumulation, gingival inflammation, carious lesions, bone loss in proximal areas, and tooth migration.[6,8,9] Likewise, very tight contact may cause periodontal complications, tooth migration, and difficulty with flossing.[7] Inadequate contact points (tight, open, or loose), can be associated with proximal caries formation.[10] Clinically, the adequacy of the contact point is assessed by the dentist by passing a wire with a slight resistance.[5,11,12] This is a simple method but does not allow evaluation of proximal contact force variations if proximal contact force is considered a physiological entity of multifactorial origin.[5,13,14] Nylon and Teflon floss materials tested in clinical studies of natural teeth found that contact forces ranged from 2 to 10 N.[15] An *in vitro* study using intact extracted permanent first premolars and measuring the contact point using waxed nylon dental floss found that the proximal contact forces ranged from 10 to 50 N.[16] Greater contact forces were described when dry surfaces were tested, and the magnitude of the forces tended to be unrelated to the contact angulation area during sliding of dental floss.[16] Thus, many studies using different devices and methodologies have tried to measure the force of the proximal contact.[17-24]

Reconstruction of a satisfactory resin composite proximal contact with correct anatomical contour and appropriate proximal contact tightness is essential but remains difficult in the placement of direct posterior restorations.[25,26] Resin composite and amalgam restoration techniques differ in proximal contact creation because of several factors, including that resin composites cannot be "condensed."[27,28] Bulk fill resin composites are gaining popularity because they reduce the number of layers during the restorative procedure

and thus the curing time.[1] Bulk fill and conventional resin composites inserted incrementally in posterior teeth have shown similar clinical performance.[29] However, proximal contact is an important clinical parameter that has not often been investigated for bulk fill resin composites. In addition, proximal contact between natural dentition and implant restorations needs to be better understood in order to facilitate faithful reproduction in posterior proximal restorations.[8]

Insufficient adaptation of the matrix to the adjacent tooth, shrinkage of material during polymerization, and position of the tooth can influence the initial proximal contact.[30,31] A clinical study of posterior resin composite restorations has already shown that contact forces do not always remain stable over time and that proximal contacts tend to diminish after a period of six months.[20] Proximal contact loss is already considered to be a complication in implant prostheses; a 7-year clinical study evaluated the effects of proximal contact loss with implants and adjacent teeth, showing that periodontal ligament displacement can occur when performing resin composite restorations.[11]

To the best of our knowledge, no study has compared the influence of simulated mechanical cycling on the proximal contact force of bulk fill resin composites. Therefore, the purpose of this *in vitro* study was to analyze the proximal contact force (N) in incremental and bulk fill class II resin composite restorations with implant molar teeth and premolars with simulated periodontal ligaments. The null hypotheses were as follows: 1) restorations created with two different resin composites have similar proximal contact force; 2) tooth condition and contact location do not influence the contact force/contact area ratio; and 3) occlusal fatigue does not reduce the proximal contact force between resin composite restorations and adjacent teeth.

METHODS AND MATERIALS

Study Design

Twenty models were made using the artificial tooth Tech Pro (IM do Brazil Ltda, São Paulo, Brazil) (protected and registered with the National Institute of Intellectual Property under P11001631-7), which was used as a base for making the replica specimens with bilateral class II occlusal-proximal (OM, occlusal-medial; and OD, occlusal-distal) standardized cavity preparations. As a control, a group without cavity preparation and without restoration was used. The specimens were restored with two protocols, bulk filling and incremental filling techniques. The number of specimens was based on the coefficient of variability and the sample calculation. The designated power of the test was 80%, with a minimum detectable difference of 20%. There was a residual standard deviation of 15% and a significance level of 0.05; these calculations resulted in the decision to use 10 specimens per group. The compositions of the resin composites, provided by the manufacturers, are listed in Table 1. The proximal contact openings of the specimens were determined by digital radiographic examination; the proximal contact

Table 1: *Resin Composites Used in this Study*

Material	Code	Resin Composite Type	Organic Matrix[a]	Filler[a]	Filler % Wt/Vol[a]	Manufacturer	Batch Number
Filtek Z350XT	Inc-Z350XT	Nanofilled	Bis-GMA, Bis-EMA, UDMA, TEGDMA	Silica and zirconia nanofillers, agglomerated zirconia silica nanoclusters	79/63	3M Oral Care (St Paul, MN, USA)	N652583
OPUS Bulk Fill APS	Bulk-OPUS	High-viscosity bulk fill	TEGDMA, Bis- EMA, UDMA	Silica with urethane dimethacrylate, salinized silica dioxide, salinized barium glass, YbF3	68	FGM (Joinville, Brazil)	N251017

Abbreviations: Bis-EMA, bisphenol A polyethylene glycol diether dimethacrylate; Bis-GMA, bisphenol A diglycidylmethacrylate; TEGDMA, triethyleneglycoldimethacrylate; UDMA, urethane dimethacrylate; YbF3, ytterbium fluoride.

[a]*Composition as given by manufacturers.*

areas were calculated (mm^2); the proximal contact force (N) was measured by microtensile tests; and the ratio between the proximal contact force (N) and proximal contact area (mm^2) was calculated.

Model Development

A model with metallic teeth was designed for testing the proximal contact of posterior restorations (Figure 1). The mandibular posterior arch of a mannequin (MOM, Manequins Odontológicos Marília, Marília, SP, Brazil), composed of the 2nd molar, 1st molar and 2nd premolar, was used. The alveolus was adapted to the root of the first molar, and the second premolar was sculpted with Vipflash acrylic resin (VIP, Pirassununga, SP, Brazil). A Morse taper 3.5 mm x 7.0 mm dental implant (Neodent, Curitiba, PR, Brazil) was placed as a substitute for the second molar. The matrix model was then duplicated using silicone rubber (Redelease, Barueri, SP, Brazil), and 20 models made of polystyrene resin (Cristal, Piracicaba, SP, Brazil) were replicated. A polystyrene resin cylindrical base 2.5 mm in diameter was added to the base of all the models to fit the cycling machine and the microtensile testing machine. Metal crowns were replicated from an individual wax pattern applied to the reference model and adapted for each model from a standard silicone matrix. Premolars were replicated in wax from an artificial-tooth rubber silicone mold and cast completely with nickel chrome alloy (Kromalit, Knebel Produtos Dentários, Porto Alegre, RS, Brazil) to ensure that wear only occurred on the specimen of interest. The implant crowns were cemented using dual cure resin cement (Allcem Core, F) light cured for 40s on each surface using a VALO Cordless LED light curing unit (Ultradent, Salt Lake City, USA) with an irradiance of 1400 mW/cm^2, which was verified using a MARC Resin Calibrator (BlueLight, Halifax, NS, Canada). The typodont first molar and the second metallic premolars were inserted in the alveoli with polyether impression material (Impregum, 3M Oral Care), simulating the periodontal ligament.[32]

Specimen Development and Preparation

One artificial first molar tooth (TechPro) received two, standard proximal occlusal cavity preparations (MO and OD) using a preparation machine.[33] A trained operator used a high-speed diamond bur (N.3198 bur, KG Sorensen, Barueri, SP, Brazil) under constant irrigation to prepare class II cavities 4 mm mesial/distal, 4 mm deep in the occlusal surface and 5.0 mm in the gingival box. This single tooth was duplicated after cavity preparation to create twenty replica teeth of polystyrene pigmented resin (Cristal, Piracicaba, SP, Brazil) with standardized preparations. For the control group, the replica of the artificial typodont tooth was used without cavity preparation, simulating an intact tooth and standardizing the position of the adjacent teeth.

Restorative Procedure

The cavities in the specimens were then cleaned with 0.12% chlorhexidine, the well was dried, and the

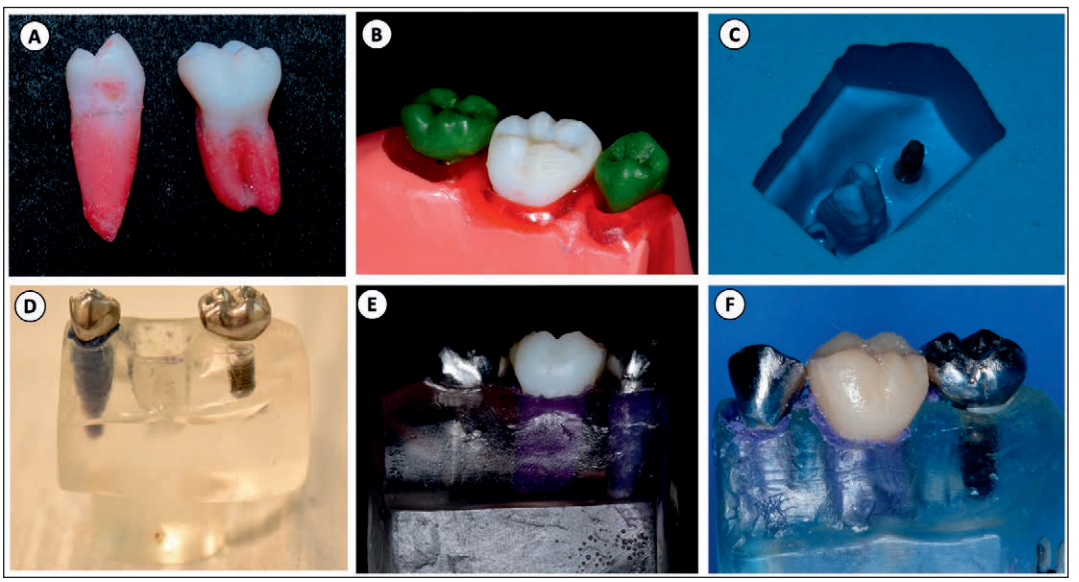

Figure 1. *Device developed for proximal contact test: (A): Artificial teeth with the periodontal ligament space simulation. (B): Artificial teeth fitted in the base model after alveoli relined with red acrylic resin shaping the roots of artificial teeth with periodontal ligament space simulation. (C): Mold base made with rubber silicone and implant position. (D): Aspect of the model with metal teeth and metal crown in position. (E): Final aspect of the model with restored specimen. (F): Final aspect of the model with intact specimen.*

adhesive system Âmbar APS (FGM) was applied. The adhesive system was photoactivated for 10 seconds. The partial preshaped metal matrix (Unimatrix, TDV Dental, Pomerode, SC, Brazil) was inserted and burnished to better define the proximal contact, and wood wedges (Cunhas anatômicas, TDV Dental) were inserted. The specimens were randomized (random.org.) and divided into two groups (n=10) according to the restorative techniques used (since the control group did not receive restorative intervention): In the Inc-Z350XT group, the proximal boxes were restored in two increments using nanofilled resin composite, Filtek Z350 XT (3M Oral Care).[34] The OPUS group was restored with a single increment of a bulk fill high viscosity resin composite, Opus Bulk Fill APS (FGM). The resin composites were photoactivated for 40 seconds. All the restorative procedures were performed by the same operator. The finishing was performed with intermittent water spray, using diamond burs (2135F and 2135FF, KG Sorensen) to remove the excess. The polishing was performed using Sof-Lex Pop-On discs (3M Oral Care).

Proximal Contact Force Calculation—Initial

The specimens were tested in a microtensile test machine (Microtensile ODEME) using a 1 mm/min crosshead speed to calculate the proximal contact force of the molar and premolar teeth. For the test, two metallic accessories were created for the microtensile machine, one for positioning the model during the tests and the other for stabilizing the dental floss during the tensile tests (Figure 2). Waxed texturized nylon dental floss with 0.09 mm diameter (Hillo, Aperibé, RJ, Brazil),[16] was inserted below the proximal contact area and was attached to the accessory stem fixed on the microtensile machine. The initial proximal contact tensile force values in newtons (N) were measured 6 times for each specimen, with 3 measurements at each proximal contact, and the average of the maximum tensile force was calculated for each proximal contact surface. The dental floss used was changed after each test, eliminating possible influence of dental floss wear on the measured proximal contact forces.[16]

Digital Radiographic Examination—Initial

Digital phosphor plate sensor radiography (Dürr Dental) was obtained within 20 cm of the source of a Timex 70 E X-ray machine (Gnatus, Ribeirão Preto, SP, Brazil). Interproximal radiography was performed, and the images were transferred from the phosphor plate to the computer by means of a scanner (VistaScan Mini View, Dürr Dental). When the specimens did not present contact between the adjacent teeth in the initial radiographic analysis, ie, when they presented visible gaps between adjacent teeth, the restored specimens were replaced because it was necessary and mandatory to start from the existing contact point to assess the contact force.[15]

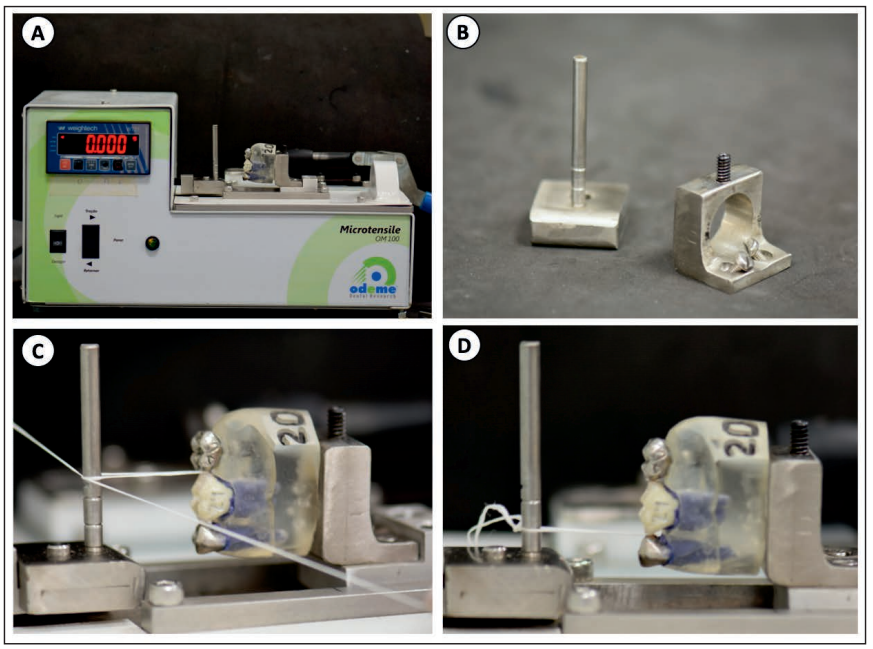

Figure 2. *Microtensile method used for contact force measurement: (A): Microtensile machine with specimen and devices positioned for measurements. (B): Devices developed to standardize the specimen position during test. (C): Clinical dental floss passed parallel around the contact point and pulled by the equipment in molar contact. (D): Dental floss passed in premolar contact.*

Mechanical Cycling Tests

The restored specimens were submitted to occlusal mechanical fatigue simulating 5 years of oral aging. The specimens were submerged in water at approximately 37°C, simulating chewing and mouth temperature and cycled 1,200,000 times from 0 to 50 N axial compressive loading with 8.0 mm diameter stainless steel spheres on the occlusal cusps with a 2 Hz frequency (Biocycle, Biopdi, São Paulo, SP, Brazil).[35,36]

Post Mechanical Fatigue Tests

After mechanical aging, the proximal contact tensile force values (N) were measured three times for each contact and six times for each specimen, and the average of the maximum tensile force was calculated as described above. The difference between the proximal contact forces was calculated: ΔPCforce = Final PCforce – Initial PCforce.

Contact Area Measurement and Calculation of Contact Force by Contact Area

The proximal contact mesio-distal and occlusal-cervical dimensions were measured using a digital caliper (Mitutoyo, Tokyo, Japan) for all restored and intact groups. The ratio between force (N) divided by area (mm^2) was calculated for all specimens in order to correlate the contact force between premolar and molar teeth.

Final Digital Radiographic Examination

Final X-ray images were taken of all the specimens, following the initial method. The initial and final X-ray images were displayed in PowerPoint (Microsoft Office, Microsoft, Washington, USA) on a screen without any manipulation or adjustment of the images. The X-ray images were blindly evaluated by two experienced and calibrated professionals, and these professionals analyzed the proximal contact using the following scores: (1) perfect proximal contact—no visible gap between restoration and adjacent tooth; (2) acceptable proximal contact—minimal areas of gapping that do not compromise the contact with the adjacent tooth; (3), unacceptable proximal contact—visible gapping between restoration and adjacent tooth that compromises the function of proximal contact (Figure 3).

Statistical Analysis

The contact force and the contact area data were tested for normal distribution (Shapiro-Wilk test) and equality of variances (Levene test), followed by parametric statistical tests. One-way ANOVA was used for comparing the initial contact force between restored and intact teeth. Two-way ANOVA followed by Tukey test was performed for contact area data and also for the contact force/contact area ratio with molar and premolar teeth. One-way repeated measurement ANOVA followed by Tukey test was performed for contact force for each tooth contact location. All the tests used $\alpha = 0.05$ as the 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). The X-ray analyses were described by the frequency.

RESULTS

Proximal Contact Force

The initial proximal contact forces (N) between the restored molars and intact typodont teeth with adjacent molars and premolars measured by microtensile tests are shown in Figure 4. One-way ANOVA revealed no significant difference between intact tooth and restored teeth using incremental (p=0.101) and bulk fill filling (p=0.198) techniques.

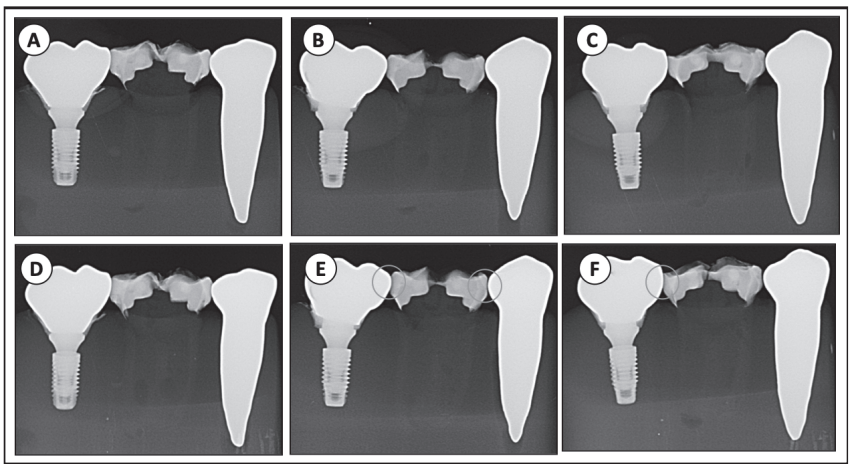

Figure 3. *Digital radiography examination: (A) and (D): Bulk-OPUS specimen score 0, with no difference at both proximal contacts comparing pre- and post-fatigue. (B) and (E): Inc-Z350XT specimen score 1, difference without continuity in both proximal contact points. (C) and (F): Bulk-OPUS specimen score 2 at the mesial and score 0 at distal, representing no difference.*

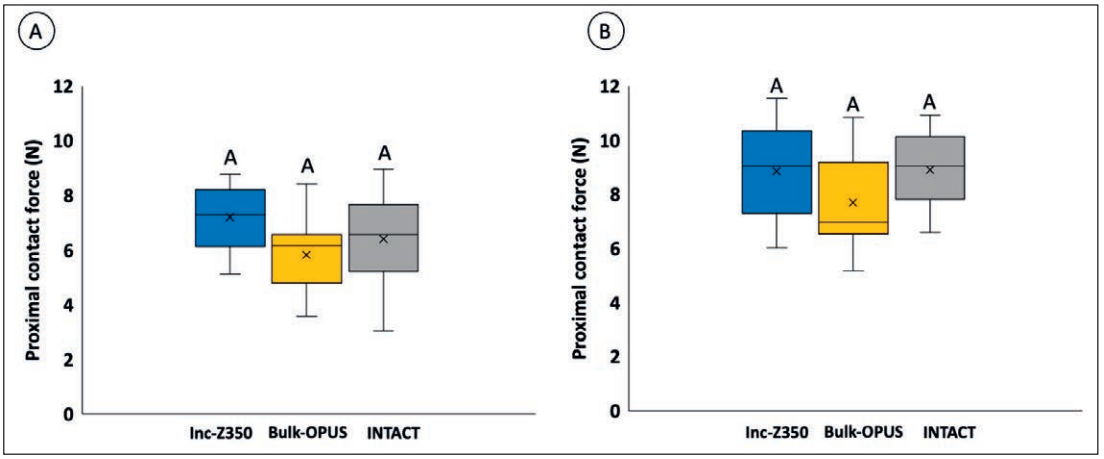

Figure 4. *The initial proximal contact forces (N) measured by microtensile tests between the restored molars and intact typodont teeth: (A): Adjacent molars. (B): Adjacent premolars. Same uppercase letters indicate no significant difference between restored and intact teeth for molar and premolar contact location, calculated using Tukey test (α=0.05).*

The proximal contact forces (N) between the restored molars (incremental and bulk fill filling technique groups) with adjacent molars and premolars, before and after the aging process are shown in Table 2. One-way repeated ANOVA revealed no significant influence for the resin composite type (p=0.102). Fatigue resulted in a significant reduction in the proximal contact force (p<0.001) irrespective of restorative material tested for both molar and premolar contact locations.

Contact Area Measurement and Calculation of Contact Force by Contact Area

The proximal contact area (mm^2) between the intact and restored molars using incremental and bulk fill filling techniques with adjacent molars and premolars are shown in Figure 5A. Two-way ANOVA revealed that the contact area was significantly influenced by contact region (p<0.001); however, no significant influence was observed for the tooth condition type (p=0.198) or for the interaction between the contact region and the tooth condition (p=0.219). The contact area measured in the molar contact region was significantly higher than the area measured in the premolar contact, irrespective of the tooth condition.

The ratios between proximal contact forces (N) and proximal contact area (mm^2) for intact and restored molars using incremental and bulk fill filling techniques with adjacent molars and premolars are shown in Figure 5B. Two-way ANOVA revealed no significant influence of contact region (p=0.248), of the tooth condition (p=0.265), or of the interaction between the contact region and tooth condition (p=0.652). The proximal contact force/proximal contact area ratio values were similar for all tested groups.

Proximal Contours—Digital Radiographic Examination

The results of the digital radiographic examination analysis are shown in Table 3. Perfect proximal contact (score 1) was predominant for the proximal contour after the fatigue mechanical cycling tests, regardless of the region analyzed (molar or premolar) or resin composite tested. The Inc-Z350XT group had three molar specimens and four premolar specimens with a

Table 2: *Means and Standard Deviations of Proximal Contact Force (N) Measured Using Microtensile Test*[a]

Restorative Material	Molar		Premolar	
	Initial	Post-fatigue	Initial	Post-fatigue
Incremental – Filtek Z350XT	7.7 ± 1.9 Aa	5.7 ± 2.4 Ba	5.8 ± 2.5 Aa	4.1 ± 1.8 Ba
Bulk fill – Opus Bulk Fill APS	8.9 ± 2.3 Aa	7.3 ± 1.9 Ba	6.1 ± 2.2 Aa	4.5 ± 2.1 Ba

Abbreviation: N, newton.

[a] *Different letters indicate a significant difference calculated using Tukey test (p<0.05); uppercase letters were used for comparing fatigue effect (pre- and post-fatigue); lowercase letters were used for comparing restorative material (Z350XT or OPUS).*

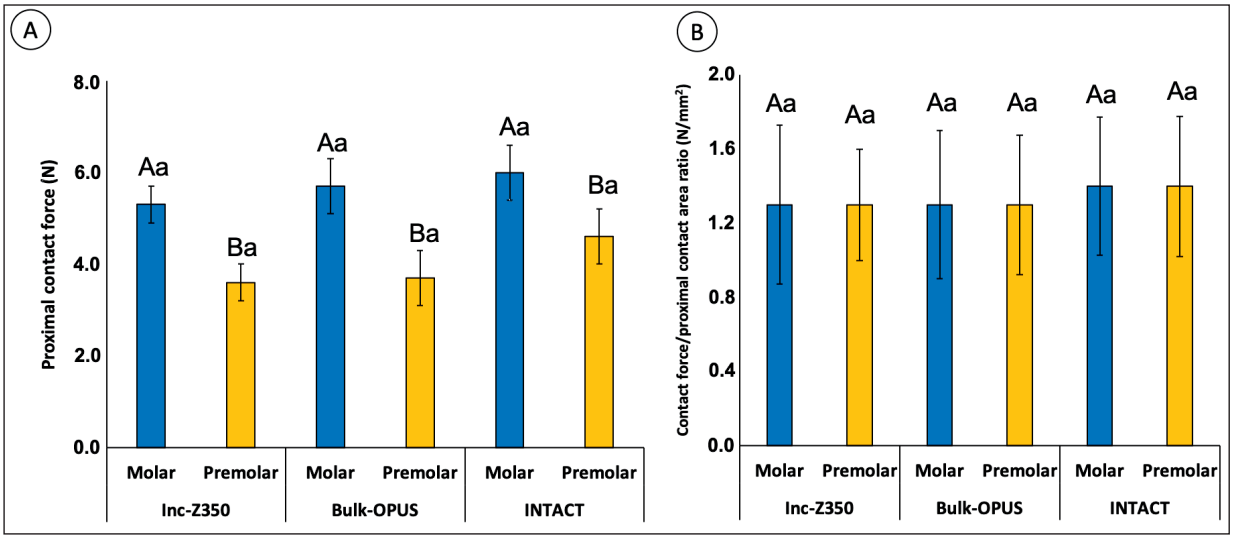

Figure 5. *(A): The proximal contact area (mm²) between the intact and restored molars using incremental and bulk fill filling techniques with adjacent molars and premolars. (B): The ratio between proximal contact forces (N) and proximal contact area (mm²) for intact and restored molars using incremental and bulk fill techniques with adjacent molars and premolars. Different letters indicate a significant difference calculated using Tukey test (α=0.05); uppercase letters were used for comparing fatigue effect (molar and premolar); and lowercase letters were used for comparing tooth condition (Inc-Z350XT, Bulk-OPUS or INTACT).*

score of 2, and no specimen had unacceptable proximal contact. The OPUS group had four molar specimens and three premolar specimens with a score of 2 and one molar specimen and one premolar specimen with a proximal contact score of 3.

DISCUSSION

The posterior restorations created using the incremental and bulk fill techniques had similar proximal contact forces; therefore, the first null hypothesis was accepted. Similar contact force/contact area ratios were verified for intact or restored teeth using the incremental and bulk fill techniques and also for implant retained molar teeth or premolar teeth with simulation of the periodontal ligament; therefore, the second null hypothesis was accepted. However, occlusal fatigue simulating five years of aging significantly decreased the proximal contact force irrespective of the region of contact with the implant retained tooth or the tooth with periodontal ligament simulation; therefore, the third null hypothesis was rejected.

Assessing proximal contact force in *in vitro* studies offers the possibility of standardizing the study conditions. Among the studies addressing proximal contact that report wear or force, many use pre-fabricated mannequin models or prepared typodonts but lack a simulation of the periodontal ligament for tests.[8,18-20,37] For this reason, in the present study we developed a model to approximate oral physiological conditions and performed a simulation of the periodontal ligament using polyether impression material.[32] The model in this study showed that proximal contact wear tends to occur only on the resin composite because both the crown on the implant retained tooth and the simulation of the natural tooth were made with metal.[8,37] To evaluate the influence of the absence of flexibility on proximal contact with adjacent teeth,

Table 3: *Proximal Contour Scorea Analysis with Digital Radiography Examination After Fatigue Mechanical Cycling*

Restorative Material	**Number of Teeth with each Criterion Evaluated[a]**					
	Molar (D)			**Premolar (M)**		
	1	**2**	**3**	**1**	**2**	**3**
Incremental – Filtek Z350XT	7	3	0	6	4	0
Bulk fill – Opus Bulk Fill APS	5	4	1	6	3	1

Abbreviations: D, distal; M, mesial.
[a] 1, perfect proximal contact; 2, proximal contact acceptable; 3, unacceptable proximal contact.

the implant retained-tooth was included in the model, simulating a frequent clinical condition.

In one study, only the initial pre-restoration and post-restoration proximal forces were clinically measured, and there was no further clinical follow-up.[22] A 7-year clinical follow-up study observed natural tooth wear associated with a dental implant but with no quantification of the proximal contact force.[11] In this study, the use of a specimen replica of the intact typodont tooth without preparation or restoration attempted to simulate the pre-restorative condition. All groups had similar contact forces, demonstrating the standardization of the specimen preparation and the effectiveness of both restorative techniques. In a similar way, the use of the single prepared tooth replica allowed us to standardize the restorative procedure among groups. It was possible to compare the proximal contact forces on different materials, with implant retained teeth and teeth with simulated periodontal ligament, and also before and after mechanical cycling.[18] This is different from *in vivo* studies where the variability of anatomical characteristics of the proximal contact between individuals challenges the evaluation interpretation before and after the restorative procedure.[14,19]

The proximal contact force device developed at the University of Technology Delft in the Netherlands used a 0.05 mm thick metal strip inserted interdentally from the occlusal surface, quantifying the proximal contact force (N) when the strip was slowly removed in the occlusal direction.[19,21-24] The methodology for measuring the proximal contact force was modified in the present study. Dental floss was used to eliminate the influence of the strip on adjacent teeth without changing the physiological conditions.[14] Additionally, the method with dental floss used in this study is similar to a method used in clinical studies.[12,15] The use of dental floss in the *in vitro* tests approximates the clinical situation, and the measured force without pre-loading can be easily translated to clinical recommendations. A clinical study using nylon and Teflon floss materials in natural teeth showed contact forces ranging from 2 to 10 N.[15] The contact-point force values found in this study, measured with waxed nylon dental floss showed similar values, ranging from 4.1 to 8.9 N, including in the control group.

In vivo studies have shown that the loss of proximal contact structure tends to be greater in the mesial area due to mesial displacement of natural teeth by the anterior component of the occlusal force.[11] Using a model that simulates the clinical situation by positioning specimens according to the natural *in vivo* position, the application of occlusal loads during the mechanical fatigue tests led to a reproduction of these forces. It might initially be expected that the higher proximal contact force between implant retained molar teeth compared with premolar teeth simulating the periodontal ligament could be explained by the lack of mobility of the implant. However, after measuring the proximal contact area, which is significantly higher in the molar-molar than in molar-premolar proximal contact, and correlating these data with the proximal contact force, the ratio values were similar for all groups, demonstrating that higher contact area generates higher contact force regardless of whether teeth are restored or intact.[38]

Occlusal fatigue resulted in a significant reduction in proximal contact force irrespective of region analyzed or restorative material tested. The reduction in the proximal contact force can be attributed to wear resulting from the restored molar intrusion process during occlusal fatigue. The tooth moved up and down, generating friction with the adjacent metallic teeth, resulting in resin composite wear. The standardization of the load application is important in order to isolate the effect generated from that of additional factors and contribute to the possible reproducibility of this methodology. The fact that the load was applied only to the restored tooth can be considered a limitation of this study but can also be considered as the worst-case scenario of proximal contact restoration. Future studies analyzing proximal wear caused by occlusal loading in all posterior teeth are necessary to complement the results of this study. However, the occlusal fatigue reduction of the proximal contact force can be correlated with the observations extracted from clinical studies, since to the best of the authors' knowledge, no *in vitro* studies have evaluated the wear of the contact point after simulation of years of aging. Clinical studies have shown that proximal contact loss occurs over time, from short follow-ups of 3 months to longer periods of up to 5 years, under different clinical conditions.[11,12,21,39]

The initial proximal contact forces were similar for intact typodont teeth and restored teeth with both restorative techniques; this finding confirmed that the restorations performed using this experimental model were effective. The incremental filling technique using Inc-Z350XT presented a contact force similar to that of the bulk filling technique using OPUS. These results occurred because Opus Bulk Fill APS and Filtek Z350XT have similar compositions for both the organic and the inorganic matrix and thus similar mechanical properties and wear resistance.[40,41] Although a resin composite is conventionally inserted in oblique increments of up to 2 mm and bulk fill is inserted in a single application, both have high viscosity

and similar inorganic filler content (Table 1). The wear of resin composite is highly dependent on the size, volume, and quantity of charged particles.[42] A previous study demonstrated that high-viscosity bulk fill resin composites had Knoop hardness number values similar to those of conventional resin composites.[43]

The results of the present study corroborate those of another study that found that proximal contact force is related to the consistency of the restorative material and the restorative technique used,[17] reaffirming that high viscosity resins produce strong proximal contact.[8] Previous studies had affirmed that conventional resin composite inserted in bulk filling did not improve proximal contact force values.[17,44,45] The X-ray image analysis showed differences before and after cycling, but similar behavior was maintained between the two groups tested, reinforcing the similarity between incremental and bulk filling techniques. The use of only one bulk resin composite should be considered as another limitation of this study. Different materials that present different filler content and mechanical properties can perform differently. [34,37,44]

Food impaction caused by the lack of proximal contact between adjacent teeth can lead to problems such as tooth movement and biofilm formation, increasing the risk of secondary caries and periodontal disease.[8,46] In contrast, very tight contact points can cause patient discomfort, make it difficult to floss, and cause periodontal injuries because the teeth are probably invading the interdental papilla space.[9] Clinical studies have shown that contact loss occurs over time at different intensities for restorations.[11,12,21,39] An annual failure rate of 2.8% has been observed for resin composite restorations in a study in which one factor evaluated was the lack of contact points and overhang.[47] For this reason, the generation of a proximal contact with adequate form and function with respect to physiological characteristics is essential for the longevity of resin composite restorations, as is expected for natural teeth[20] and implant prostheses.[11,12] The use of bulk fill resin composite provided good performance in this regard; however, future clinical studies should be performed.

As observed with the new methodology tested, occlusal fatigue was supported only by the central tooth, and the effect of fatigue may have been influenced by the adjacent tooth type, in turn influencing the results. Future studies varying the viscosity of resin composites, using a larger number and different compositions of bulk fill resin composites; and using specimens with greater resistance to the effects of cycling are needed to complement the current findings, leading to *in vitro* analysis of contact-point strength closer to clinical conditions.

CONCLUSIONS

Within the limitations of this *in vitro* study design and considering the restorative materials tested, the following conclusions can be drawn:

- Occlusal fatigue simulating an aging process of 5 years decreased the proximal contact force between the implant and adjacent teeth with simulated periodontal ligaments.
- The proximal contact forces before occlusal fatigue were similar for both restorative techniques compared with the intact-tooth control group.
- The proximal contact area was significantly larger for the molar-molar than for the molar-premolar location; however the ratio of proximal contact force/contact area were similar for all tested groups.
- Bulk filling using high viscosity Opus bulk fill APS resin composite showed similar proximal contact forces before and after occlusal fatigue to the incremental filling technique performed with Filtek Z350.

Conflict of Interest

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

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Effects of Hydrothermal Treatment on the Phase Transformation, Surface Roughness, and Mechanical Properties of Monolithic Translucent Zirconia

CY Zhang • C Agingu • H Yang • H Cheng • H Yu

Clinical Relevance

Although the mechanical properties of translucent monolithic Y-TZP ceramics were found to be related to the coloring and material types, minimal effects of hydrothermal treatment on their phase transformation, surface roughness, and mechanical properties were observed.

SUMMARY

Objectives: This study aimed to investigate the effects of hydrothermal treatment on four types of monolithic, translucent, yttria-stabilized, tetragonal zirconia polycrystals (Y-TZPs).

Methods and Materials: Two commercially available Y-TZP brands—SuperfectZir High Translucency (Aidite Technology Co, China) and Katana HT (Kuraray Noritake Dental, Japan) were assessed. For each brand of Y-TZP, materials

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of four coloring types, including noncolored (NC), colored by staining (CS), precolored (PC), and multilayered (ML) specimens were investigated after hydrothermal aging in an autoclave at 134°C/0.2 MPa for 0 (control group), 5, 10, and 20 hours. The tetragonal-to-monoclinic phase transformation, surface roughness, flexural strength, and structural reliability (Weibull analysis) were measured and statistically analyzed (α=0.05). The subsurface microstructure was analyzed with scanning electron microscopy.

Results: The group ML exhibited the lowest flexural strength and Weibull characteristic strength among the four coloring types (p<0.05). Slight increases in the monoclinic phase volume, flexural strength, and Weibull characteristic strength were observed after hydrothermal aging (p_{all}<0.05). Regardless of coloring type, no significant effects of aging on the Weibull modulus or surface roughness were found for the tested materials. Compared with the Katana HT cross-sections, the SuperfectZir High Translucency cross-sections exhibited a similar but thicker transformation zone.

Conclusions: The coloring procedure and material type were found to affect the mechanical properties and aging resistance of translucent monolithic Y-TZP ceramics. Regardless of the aging time, the surface roughness of the tested Y-TZP ceramics remained unchanged.

INTRODUCTION

With the development of computer-aided design–computer-aided manufacturing (CAD–CAM), monolithic restorations are increasingly being promoted to avoid the cohesive fracture of veneering porcelain (chipping) and facilitate a more conservative tooth preparation.[1,2] Zirconia has been a dominant ceramic, typically fabricated in monolithic form, for a wide range of clinical applications due to its excellent mechanical properties.[3-5] At ambient pressure, zirconia has three crystalline phases: tetragonal (*t*), monoclinic (*m*), and cubic (*c*).[6,7] For biomedical applications, *t*-phase zirconia is typically stabilized by adding yttria (Y_2O_3) to pure zirconia to achieve enhanced mechanical properties, which is known as yttria-stabilized tetragonal zirconia polycrystal (Y-TZP).[8,9] Among all the available dental Y-TZP ceramics, three mol% (5.2 wt%) yttria is the most common dopant for stabilizing zirconia and is denoted as 3Y-TZP. However, the major drawback of the first generation of 3Y-TZP is its high opacity, especially when compared with glass ceramics.[7] To reduce the opacity of Y-TZP, various approaches have been proposed, including the reduction of an alumina additive, the elimination of porosity, an increase in the sintering density, and an increase in the *c*-phase content.[7,9-11] The *c*-phase of zirconia is isotropic in different crystallographic directions, which decreases light scattering at grain boundaries. The *c*-phase attracts yttria and leaves the *t*-phase prone to spontaneous transformation. Therefore, researchers have attempted to stabilize pure zirconia with a significant amount of *c*-phase by increasing the yttria content.[12] The latest translucent Y-TZP contains a higher concentration of yttria (4Y-TZP or 5Y-TZP) than that of 3Y-TZP, and they exhibit comparable translucency with lithium disilicate.[7,9,10] These materials are typically used for anterior crowns and fixed dental prostheses (FDPs). Improved translucency of 5Y-TZP and 4Y-TZP has been achieved by increasing the amount of transparent-phase (*c*-phase) zirconia; however, the mechanical strength decreases in this case.[10,13] A dental laboratory survey of 39,287 restoration records reported a failure rate of 2.06% over 5 years for anterior monolithic restorations.[14] The second generation of 3Y-TZPs (translucent 3Y-TZPs), which have been refined by reducing the amount of alumina additive and decreasing the grain size and sintering porosity, has been considered to strike a suitable balance between esthetics (color and translucency) and function (strength).[7,9,15] From a clinical point of view, translucent 3Y-TZPs are the most widely used, and they are considered the proper dental ceramic for monolithic restorations.[7,16,17]

Conventionally, Y-TZPs are produced with uniform translucency and color (single-layered).[18] To change the basic color of Y-TZP (white to ivory) to a natural tooth color, two main approaches for coloring Y-TZP are available: precoloring and coloring by staining.[19-21] For precolored Y-TZP, metal oxides capable of reproducing a color are added into the starting powder. The colored-by-staining technique involves dipping the noncolored Y-TZP in coloring liquids or painting the coloring liquids on with a brush. These two coloring techniques have been proven to successfully reproduce the color of human teeth, although conflicting results have been reported regarding the effects of coloring procedures on the flexural strength of Y-TZPs. Ban and others[18] observed a reduction in the flexural strength and fracture toughness of Y-TZPs colored by staining in a coloring liquid containing erbium (Er) and neodymium (Nd) ions. In contrast, other studies reported that the coloring procedure exhibited no effects on the flexural strength of Y-TZPs.[6,22,23] The lack of consensus may be due to the different Y-TZPs and coloring liquids adopted

in previous studies. Recently, multilayered Y-TZPs with gradual changes in color and translucency have been introduced commercially. Multilayered Y-TZPs normally include three to four layers (enamel, dentin, and transition layers) corresponding to the transition from enamel to dentin.[9,22] Although manufacturers claim that multilayered Y-TZP blocks have a flexural strength similar to that of conventional single-layered products,[24] some authors have reported results that conflict with the internal data of these manufacturers.[13,15] Consequently, thorough investigations are additionally needed to provide clinical recommendations.

Theoretically, all types of 3Y-TZPs undergo a *t*- to *m*-phase transformation when exposed to stress in the oral environment.[7,25,26] The phase transformation leads to a volumetric expansion that can stop crack propagation and results in the superior mechanical properties of 3Y-TZPs (transformation toughening). However, a slow *t*- to *m*-phase transformation can also occur without stress under moist conditions and at body temperature, which is referred to as low-temperature degradation (LTD) or aging.[27,28] Although limited evidence of LTD is available in clinical situations, LTD leads to a slow phase transformation, which reduces the flexural strength of 3Y-TZPs under laboratory settings.[3,16] The effects of different coloring procedures (precoloring vs coloring by staining) on the aging behavior of 3Y-TZPs have been investigated in a previous study.[19] After hydrothermal aging, the precolored 3Y-TZP exhibited significantly greater phase transformation than the colored-by-staining 3Y-TZP. Given that a small deviation in composition, structure, and fabrication can make a huge difference,[9,25,29] it is critical to evaluate and compare the mechanical and physical properties of different types of 3Y-TZPs after aging in a comprehensive manner. However, to the best of the authors' knowledge, limited information is available.

Therefore, this study aimed to evaluate the surface roughness, subsurface microstructure, flexural strength, structural reliability (Weibull analysis), and *t*- to *m*-phase transformation of four coloring types of second generation 3Y-TZPs (noncolored, precolored, colored by staining, and multilayered) produced by two commercially available brands (SuperfectZir High Translucency and Katana HT). The null study hypotheses were as follows: 1) that the four coloring types of 3Y-TZPs would exhibit similar mechanical and physical properties, and 2) that the four coloring types of 3Y-TZPs would behave similarly after hydrothermal aging.

METHODS AND MATERIALS

Specimen Preparation

Two commercial brands of translucent 3Y-TZPs (SuperfectZir High Translucency, Aidite Technology Co, Qinhuangdao, China; Katana HT, Kuraray Noritake Dental, Niigata, Japan) were investigated in the present study. For each brand, four coloring types of products were employed: noncolored (NC), colored by staining (CS), precolored (PC), and multilayered (ML). The characteristics of the investigated 3Y-TZPs are listed in Table 1.

Table 1: *Characteristics of the Materials Used in this Study*

Material	Type of Product	Chemical Components (wt%)	Manufacturer	Sintering Conditions		
				Heating	Dwelling Time	Cooling
SuperfectZir High Translucency	NC	ZrO_2+HfO_2:92%-96%; Y_2O_3: 5.3%; Al_2O_3: 0.25 wt%	Aidite Technology Co., Qinhuangdao, China	7°C/min to 900°C + 5°C/min to 1530°C	2 h	10°C/min to room temperature
SuperfectZir High Translucency A2	PC					
SuperfectZir High TranslucencyMultilayer	ML					
Katana HT10	NC	ZrO_2+HfO_2:90%-95%; Y_2O_3: 5%-6%; other oxide: 0-2 wt%	Kuraray Noritake Dental, Niigata, Japan	10°C/min to 1500°C	2 h	10°C/min to room temperature
Katana HT12	PC					
Katana HTML	ML					

Abbreviations: ML, multilayered; NC, noncolored; PC, precolored.

Bar-shaped specimens were fabricated using a CAD–CAM milling machine (Zenotec, Wieland, Germany) from green-stage discs with compensation for shrinkage during dense sintering.[19] ML specimens were fabricated using ML discs with enamel and transition layers. During fabrication, half of the ML specimen (1.5 mm in thickness) was within the enamel layer, while the other half was within the transition layer. All four long edges of each specimen were chamfered according to ISO 14704:2016.[30] The four surfaces of the specimens were wet-polished with 600-grit and 1200-grit silicon carbide discs (Buehler, Lake Bluff, IL, USA) and ultrasonically cleaned for 15 minutes. CS specimens were colored by dipping NC specimens into a coloring liquid (zirconia coloring liquid A2, Aidite Technology Co) for 2 minutes at room temperature. For the PC and ML specimens, an A2 shade was selected. All specimens were sintered according to the respective instructions of the manufacturers. After sintering, the four surfaces of the specimens were wet polished using a polishing device (PG-1S, Biaoyu Co, Shanghai, China) with 1000-grit and 2000-grit silicon carbide discs to control the final dimensions with tolerances of ±0.01 mm (length 22 mm, width 4 mm, and thickness 3 mm).

Aging Treatment

An LTD process was simulated using hydrothermal aging, according to ISO 13356:2016.[31] The specimens of each type of 3Y-TZP were randomly divided into four groups based on aging duration (n=37). The aging treatment was performed using an autoclave (HE-50, Hirayama, Japan) at 134°C in a water vapor atmosphere at 0.2 MPa for 0 (control), 5, 10, and 20 hours.

Phase Transformation Analysis

The crystalline phases of the specimens from each group (n=5) were analyzed using an X-ray diffractometer (Empyrean, PANalytical, the Netherlands). The X-ray diffraction (XRD) patterns were recorded at 40 kV (generator voltage) and 40 mA (tube current) with Cu Kα radiation. The 2θ scan range was between 26° and 36°, with a step size of 0.01° and a scan time of 18.9 seconds/step. The monoclinic phase fraction (X_m) was calculated according to the equation proposed by Garvie and Nicholson[32]:

$$X_m = \frac{I_m(-111) + I_m(111)}{I_m(111) + I_m(-111) + I_t(101)} \quad (1)$$

where $I_m(-111)$ and $I_m(111)$ represent the monoclinic peak intensities at 2θ=28.2° and 31.4°, respectively, and $I_t(101)$ represents the tetragonal peak intensity at 2θ=29.9°.

The monoclinic volume fraction (V_m) was then calculated using a method described by Toraya and others[33]:

$$V_m = \frac{1.311X_m}{1 + 0.311X_m} \quad (2)$$

Surface Roughness Measurement

The surface roughness of the specimens was measured using a stylus profilometer (SEF 680, Kosaka Laboratory, Japan) after the respective aging treatment (n=30). The profilometer was used with a diamond stylus that moved along a length of 2.5 mm at a speed of 0.5 mm/s with a cut-off value of 0.8 mm. Four readings were taken on a single selected surface of each specimen, and the average R_a values were calculated for statistical analysis.

Flexural Strength Measurement

After the surface roughness measurement, a four-point flexure test was performed using a universal testing machine (Instron 1186, Instron, London, United Kingdom) at a crosshead speed of 1 mm/minute (n=30). The flexural strength (σ) was calculated using the following equation[34]:

$$\sigma = \frac{3Pl}{4Wb^2} \quad (3)$$

where P is the loading load, l is the length of the test span, and w and b are the width and thickness of the beam specimen, respectively.

Scanning Electron Microcopy (SEM) Observation

The cross-sectional topography patterns were examined using a scanning electron microscope (Quanta 250, FEI, USA). For each coloring type of Y-TZP ceramic, two specimens were cross-sectioned. The sections were mounted on aluminum stubs and sputter-coated with gold before being examined at an acceleration voltage of 15 kV. The images were taken at a magnification of 5000×. The depth of *t*- to *m*-phase transformation zone was estimated as described in a previous study.[35]

Energy Dispersive X-ray Spectroscopy (EDS) Measurement

EDS analysis was performed to measure the yttria contents (expressed as weight percentages) of the Y-TZPs using the same SEM instrument equipped with an energy-dispersive X-ray spectrometer. For each coloring type of Y-TZP ceramic, two green-stage

specimens were fabricated for EDS measurement. For the group ML, EDS measurements were performed in each layer (enamel and transition).

Statistical Analysis

The statistical analysis was performed with the SPSS statistical software package (SPSS 19.0 for Windows, SPPS, Chicago, IL, USA). The Shapiro–Wilk test confirmed the normal distribution of the data. Three-way analysis of variance (ANOVA) with Tukey post hoc test was used to analyze the results of surface roughness, flexural strength, and V_m (α=0.05).

To determine the structural reliability, the flexural strength data were also submitted to a Weibull distribution. According to the Weibull distribution, the probability of failure (P_f) of a brittle material can be calculated with the following equation[36]:

$$P_f = 1 - \exp\left[-\left(\frac{\sigma}{\sigma_0}\right)^m\right] \quad (4)$$

where m is the Weibull modulus related to the dispersion of the failure data, σ_0 is the characteristic strength representing the stress level in which 63.21% of the specimens will fail, and σ is the strength at a given P_f. The probability of failure can be estimated from the following equation[36]:

$$PP_f = 1 - \exp\left[-\left(\frac{\sigma}{\sigma_0}\right)^m\right]_f = 1 - \frac{i}{n+1} \quad (5)$$

where i is the ranking of the strength data when arranged in ascending order, and n is the number of specimens.

Using equations 4 and 5, the Weibull modulus (m) and the characteristic strength (σ_0) of the tested materials were calculated according to a method previously described in detail.[37]

RESULTS

The yttria contents for each group ranged from 5.07 to 5.75 wt% (Table 2). The R_a values of all groups are listed in Table 3. No significant differences were found between brands, among material types, or among aging durations (p=0.215, p=0.806, and p=0.106, respectively).

The V_m values of all groups are listed in Table 4. The V_m values of all groups significantly increased with aging duration (p<0.001). There were significant differences in the V_m among the material types (ML, NC, PC>CS, p<0.001) and between brands (SuperfectZir High Translucency>Katana HT, p=0.027).

The flexural strength values of all groups are listed in Table 5. The flexural strength significantly increased with aging duration (p<0.001). There were significant differences in flexural strength among the material types (CS>PC>NC>ML, p<0.001) and between brands (Katana HT>SuperfectZir High Translucency, p<0.001). For nonaged specimens, the group CS exhibited the highest flexural strength, whereas the group ML showed the lowest flexural strength.

The Weibull modulus (m) values of all groups are listed in Table 6. The Weibull modulus ranged from 9.17 to 14.98. The Weibull modulus of all groups remained unchanged after aging. No significant differences were found in the Weibull modulus between brands. In most cases, the Weibull modulus was similar among all groups.

The characteristic strength (σ_0) values of all the groups are shown in Table 7. The characteristic strength remained unchanged after aging, except for the groups CS and NC produced with SuperfectZir High Translucency. The group ML exhibited the lowest characteristic strength among the four groups.

Similar grain sizes were observed for the control specimens of different groups. Representative cross-section images of the control specimens and specimens aged for 20 hours are shown in Figure 1. The cross-sections of aged samples revealed the existence of two distinct regions: a zone near the surface that appeared relatively rough, displaying grains with sharp edges (transformed region) and a zone similar to the unaged specimens (untransformed region). After aging for 20 hours, the depth of transformation was found to be approximately 15 μm for the SuperfectZir High Translucency specimens and 6 μm for the Katana HT specimens.

Table 2: *Means and Standard Deviations (SD) of Yttria (wt%) for Each Group*

	ML	PC	CS	NC
SuperfectZir High Translucency	Enamel layer: 5.32 (0.15) Transition layer: 5.11 (0.04)	5.14 (0.18)	5.07 (0.09)	5.12 (0.13)
Katana HT	Enamel layer: 5.58 (0.18) Transition layer: 5.44 (0.25)	5.75 (0.22)	5.64 (0.16)	5.41 (0.20)

Abbreviations: ML, multilayered; PC, precolored; CS, colored by staining; NC, noncolored.

Table 3: *Means and Standard Deviations (SD) of Ra (μm) for Each Group*[a]

Aging	**ML**	**PC**	**CS**	**NC**
SuperfectZir High Translucency				
0 hour	0.29 (0.01) Aa	0.31 (0.03) Aa	0.32 (0.01) Aa	0.31(0.02) Aa
5 hours	0.31 (0.02) Aa	0.31 (0.01) Aa	0.33 (0.05) Aa	0.31(0.05) Aa
10 hours	0.32 (0.05) Aa	0.32 (0.03) Aa	0.35 (0.04) Aa	0.31(0.02) Aa
20 hours	0.31 (0.06) Aa	0.33 (0.02) Aa	0.36 (0.05) Aa	0.33(0.07) Aa
Katana HT				
0 hour	0.28 (0.02) Ab	0.30 (0.03) Ab	0.28 (0.04) Ab	0.30(0.04) Ab
5 hours	0.31 (0.04) Ab	0.39 (0.02) Ab	0.30 (0.01) Ab	0.30(0.01) Ab
10 hours	0.28 (0.01) Ab	0.31 (0.05) Ab	0.32 (0.08) Ab	0.30(0.06) Ab
20 hours	0.34 (0.03) Ab	0.32 (0.06) Ab	0.32 (0.03) Ab	0.36(0.02) Ab

Abbreviations: ML, multilayered; PC, precolored; CS, colored by staining; NC, noncolored.
[a] *Different uppercase letters in a row indicate significant differences at each time point (*p*<0.05). Different lowercase letters in a column indicate significant differences for different groups of each tested material (*p*<0.05).*

Table 4: *Means and Standard Deviations (SD) of Vm (%) for Each Group*[a]

Aging	**ML**	**PC**	**CS**	**NC**
SuperfectZir High Translucency				
0 hour	1.37 (0.79) Aa	1.40 (0.46) Aa	1.55 (0.72) Aa	0.83 (0.29) Aa
5 hours	15.94 (0.20) Ab	15.33 (0.26) Ab	14.49 (0.65) Ab	16.28 (0.26) Ab
10 hours	30.04 (0.14) Ac	29.49 (0.17) Ac	26.99 (1.00) Bc	30.46 (0.15) Ac
20 hours	38.76 (2.51) Ad	38.89 (0.15) Ad	35.50 (0.55) Ad	38.17 (2.14) Ad
Katana HT				
0 hour	1.36 (0.25) Ae	2.19 (0.61) Ae	2.13 (0.58) Ae	2.16 (0.82) Ae
5 hours	8.71 (1.03) Af	6.31 (3.69) Af	6.48 (0.76) Af	7.29 (1.05) Af
10 hours	17.99 (0.26) Ag	18.06 (0.31) Ag	14.90 (0.18) Bg	17.59 (0.75) Ag
20 hours	26.65 (0.14) Ah	26.21 (0.20) Ah	25.48 (2.54) Ah	26.36 (0.40) Ah

Abbreviations: ML, multilayered; PC, precolored; CS, colored by staining; NC, noncolored.
[a] *Different uppercase letters in a row indicate significant differences at each time point (*p*<0.05). Different lowercase letters in a column indicate significant differences for different groups of each tested material (*p*<0.05).*

DISCUSSION

A trend toward monolithic restorations has been witnessed over recent decades. Among the available materials, zirconia, specifically Y-TZP, has gained increasing popularity, and various types have been developed for monolithic use, including single- and multiple-unit restorations and implant abutments.[7,17] As one of the top material choices, 3Y-TZP ceramics have been shown to be susceptible to LTD.[16,38] The aging process may cause grain detachment and ultimately lead to strength degradation.[7,9] However, the effects of aging on 3Y-TZPs are material dependent, and studies comparing the aging behavior of different material types show conflicting results. The present study was therefore conducted to provide knowledge for developing evidence-based material selection criteria in the context of clinical practice. Based on the present findings, the null hypotheses that the four coloring types of 3Y-TZPs would exhibit similar mechanical and physical properties and that the four coloring types of 3Y-TZPs would behave similarly after hydrothermal aging were rejected.

Table 5: *Means and Standard Deviations (SD) of Flexural Strength (MPa) for Each Group*[a]

Aging	ML	PC	CS	NC
SuperfectZir High Translucency				
0 hour	656.3 (67.1) Aa	737.3 (61.1) Ba	793.8 (59.3) Ba	756.3 (65.0) Ba
5 hours	689.0 (75.6) Aa	777.9 (78.1) Ba	842.5 (86.1) Cab	797.9 (74.3) Ba
10 hours	649.6 (61.3) Aa	751.6 (70.2) Ba	881.4 (86.8) Cab	852.0 (80.2) Cb
20 hours	695.4 (69.1) Aa	769.1 (65.0) Ba	884.6 (92.8) Cb	863.1 (74.6) Cb
Katana HT				
0 hours	849.2 (72.6) Ac	961.1 (80.2) Bc	987.6 (70.9) Bc	973.0 (79.0) Bc
5 hours	882.7 (80.9) Ac	1001.8 (71.8) Bc	996.8 (76.5) Bcd	977.0 (71.3) Bc
10 hours	898.8 (73.3) Ac	1019.9 (75.4) Bc	1034.6 (65.4) Bd	981.6 (80.5) Bc
20 hours	887.9 (88.8) Ac	1018.2 (91.9) Bc	1009.6 (73.1) Bcd	963.3 (66.8) Bc

Abbreviations: ML, multilayered; PC, precolored; CS, colored by staining; NC, noncolored.
[a] *Different uppercase letters in a row indicate significant differences at each time point (p<0.05). Different lowercase letters in a column indicate significant differences for different groups of each tested material (p<0.05).*

Table 6: *Means and 95% Confidence Intervals (95% CI) of the Weibull Modulus (m) Values for All Groups*[a]

Aging	ML	PC	CS	NC
SuperfectZir High Translucency				
0 hours	10.3 (8.9- 11.7) Aa	11.2 (8.1- 14.3) Aa	13.8 (11.8- 15.9) Aa	11.8 (10.0- 13.7) Aa
5 hours	9.4 (7.6- 11.2) Aa	9.7 (7.5- 11.8) Aa	10.1 (8.7- 11.4) Aa	11.4 (9.4- 13.4) Aa
10 hours	9.2 (6.0- 12.3) Aa	10.9 (9.4- 12.5) Aa	11.0 (10.0- 12.1) Aa	11.0 (9.7- 12.4) Aa
20 hours	9.3 (6.7- 11.9) ABa	12.5 (11.2- 13.7) Ba	10.2 (9.3- 11.1) Aa	12.1 (10.9- 13.4) ABa
Katana HT				
0 hour	11.6 (9.3- 13.9) Ab	12.0 (8.7- 15.4) Ab	14.8 (13.4- 16.2) Ab	12.2 (9.7- 14.7) Ab
5 hours	11.4 (10.2- 12.6) Ab	15.0 (12.9- 17.0) Bb	13.8 (12.2- 15.3) ABb	14.5 (13.2- 15.9) Bb
10 hours	12.8 (11.2- 14.4) Ab	13.9 (11.9- 15.8) ABb	16.9 (15.2- 18.7) Bb	13.1 (11.2- 14.9) Ab
20 hours	10.5 (9.0- 12.1) Ab	11.5 (9.7- 13.2) Ab	14.0 (11.6- 16.5) Ab	14.1 (10.8- 17.4) Ab

Abbreviations: ML- multilayered; PC- precolored; CS- colored by staining- NC- noncolored.
[a] *Different uppercase letters in a row indicate significant differences at each time point (p<0.05). Different lowercase letters in a column indicate significant differences for different groups of each tested material (p<0.05).*

The four coloring types tested in this study (PC, NC, CS, and ML) cover all the available coloring options for monolithic Y-TZP ceramics. The aging treatment was performed according to ISO 13356:2015 and previous studies.[9,25,31] Based on the estimation proposed by Cattani-Lorente and others,[26] 20 hours of accelerated aging at 134°C and 0.2 MPa is estimated to correspond to 8 years *in vivo*.

Flexural strength is an important indicator for estimating the clinical performance of dental restorations.[37,39] In the present study, the flexural strength of all the tested 3Y-TZPs ranged from 656.3 to 987.6 MPa, indicating that the tested materials can be used in monolithic forms for up to three-unit FDPs.[34] However, it is unlikely to use monolithic translucent 3Y-TZPs for the application of long-span FDPs. The ML specimens exhibited the lowest flexural strength and Weibull characteristic strength among the four tested material types. The above result was in agreement with previous studies in which a significantly lower flexural strength was found for multilayered Y-TZPs than single-layered Y-TZPs.[15,22] No significant differences were observed in

Table 7: *Means and 95% Confidence Intervals (95% CI) of the Characteristic Strength (σ_0) Values for All Groups*[a]

Aging	**ML**	**PC**	**CS**	**NC**
SuperfectZir High Translucency				
0 hour	621.3 (608.4- 634.2) Aa	700.3 (671.9- 728.7) Ba	762.1 (722.7- 791.6) Ba	721.4 (704.2- 738.6) Ba
5 hours	648.7 (632.6- 664.8) Aa	735.4 (715.5- 755.3) Ba	796.5 (783.6- 809.4) Ca	759.9 (741.0- 778.7) Bb
10 hours	611.9 (583.5- 640.3) Aa	714.0 (700.0- 728.1) Ba	837.9 (828.3- 847.5) Cb	810.0 (797.4- 822.6) Dc
20 hours	655.9 (632.4- 679.4) Aa	735.9 (724.3- 747.5) Ba	836.9 (828.3- 847.5) Cb	824.6 (813.1- 836.2) Cc
Katana HT				
0 hour	809.7 (788.3- 831.1) Ad	918.3 (946.2- 985.4) Bd	951.4 (938.3- 964.5) Bd	930.2 (906.2- 954.1) Bd
5 hours	840.8 (829.6- 852.1) Ad	965.8 (961.4- 999.3) Bd	958.2 (943.7- 972.7) Bd	941.0 (928.2- 953.8) Bd
10 hours	860.8 (845.7- 876.0) Ae	980.4 (961.4- 999.4) Bd	1001.7 (984.8- 1018.7) Be	941.1 (923.6- 958.7) Cd
20 hours	841.5 (826.6- 856.5) Ade	970.3 (953.8- 989.9) Bd	970.9 (947.3- 994.4) Bde	926.5 (895.5. 957.6) Bd

Abbreviations: ML, multilayered; PC, precolored; CS, colored by staining; NC, noncolored.

[a]*Different uppercase letters in a row indicate significant differences at each time point (*p<*0.05). Different lowercase letters in a column indicate significant differences for different groups of each tested material (*p<*0.05).*

the flexural strength among the single-layered 3Y-TZPs that were colored using different methods. The present findings and previous reports[6,21,22] suggest that the coloring liquid does not affect the flexural strength of translucent monolithic 3Y-TZPs.

The XRD analysis showed different percentages of phase transformation among the tested 3Y-TZPs that were subjected to aging. The *t*- to *m*-phase transformation increased with hydrothermal aging. The highest V_m was found in the specimens aged for 20 hours (25.5%-38.9%), which was within the same range as the previous studies.[19,40] Among the four material types, the lowest V_m value was found in the group CS for both brands tested, which was consistent with a previous study.[19] Various factors, including particle size,[41-43] sintering protocol,[44,45] yttria content,[7,9] and coloring procedure[18,19] have been attributed to the aging resistance of monolithic Y-TZPs. The relatively

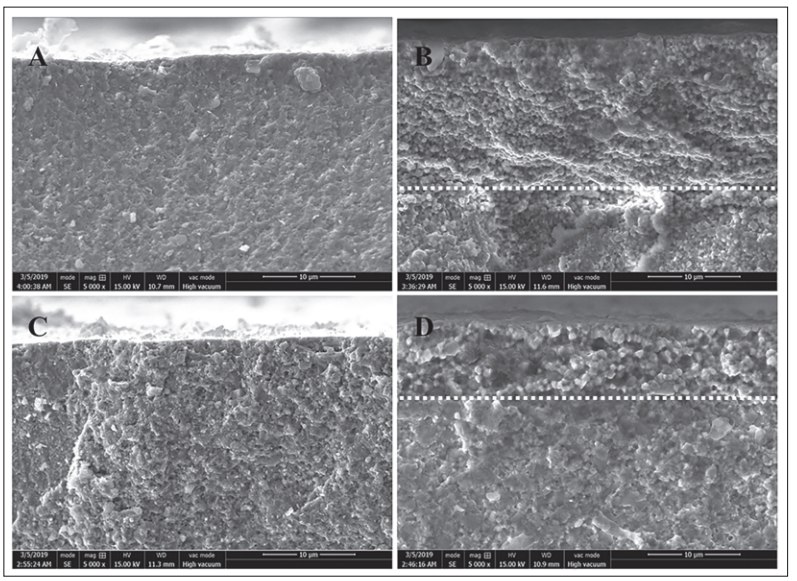

Figure 1. *Representative cross-sectional scanning electron microscope (SEM) images of the specimens at a magnification of 5000×: A. precolored specimen of SuperfectZir High Translucency aged for 0 hour (control); B. precolored specimen of SuperfectZir High Translucency aged for 20 hours; C. precolored specimen of Katana HT aged for 0 hour (control); and D. precolored specimen of Katana HT aged for 20 hours. The transformation zones are indicated by the dashed lines (B and D).*

better resistance to hydrothermal aging of the CS group may be explained by the ceria in the coloring liquid acting as a sintering aid.[19,46] Significantly, different aging resistances were also noticed between the two commercial brands, possibly due to the slightly higher yttria content in Katana HT and the different metal oxides incorporated to achieve the desired shade.[11,47] Since information about the coloring liquids and metal oxides incorporated is not available, further studies are needed to clarify these hypotheses. According to the SEM observations, the depth of transformation of the Katana HT specimens was thinner than that of the SuperfectZir High Translucency specimens (6 μm vs 15 μm), which correlated well with the V_m data and with previous studies.[19,25]

Slight but significant increases in flexural strength and characteristic strength were observed after hydrothermal aging, which was in agreement with previous studies.[40,48-50] The increase in the strength of zirconia was dependent on the percentage of the *t*- to *m*-phase transformation. The above finding indicates that the increased strength may be due to the transformation toughening mechanism, as the phase transformation on the surface of zirconia creates compressive stresses around the surface defects and further inhibits crack propagation.[51,52] However, the flexural strength values of 3Y-TZPs have also been reported to decrease[47] or remain unchanged[19,53,54] in previous studies. The conflicting findings in the literature may be due to different Y-TZP compositions, aging protocols, and flexural strength measurements. Given that only a 5% to 10% increase was found in the flexural strength of aged specimens, the present finding may not have any clinical significance. Importantly, the Weibull modulus remained unchanged after 20 hours of hydrothermal aging, indicating that the structural reliability of the tested 3Y-TZPs was stable.

Surface roughness measurements were performed as suggested by ISO 14704:2016.[30] Similar to previous reports,[16,50] aging had no effects on the surface roughness of translucent monolithic 3Y-TZPs.

The results of this study showed that the translucent monolithic 3Y-TZPs were mechanically stable and demonstrated resistance to aging; however, the mechanical properties and aging resistance were material dependent. The present study investigated specimens made from the enamel and transitional layers of multilayered zirconia. The mechanical properties and aging behavior may vary among different layers [11]. Moreover, the present findings need to be interpreted with caution, because the simulated hydrothermal aging does not include any mechanical loading that is present *in vivo*. Nevertheless, further long-term clinical studies are important for drawing solid scientific recommendations.

CONCLUSIONS

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

1. The mechanical properties of multilayered 3Y-TZP ceramics were significantly lower than those of 3Y-TZP ceramics of other coloring types.
2. The flexural strength and V_m of 3Y-TZP ceramics significantly increased after aging, while the surface roughness remained unchanged.

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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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Effect of Time and Temperature of Air Jet on the Mechanical and Biological Behavior of a Universal Adhesive System

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Clinical Relevance

Insufficient polymerization of resinous materials increases the toxicity of these products and results in the formation of a more fragile polymer. The application of hot air jet blast favors the polymerization of the material, reducing its cytotoxicity and increasing its adhesion to dentin.

SUMMARY

Objectives: To evaluate the influence of heat application on the degree of conversion (DC) of the 3M Single Bond Universal Adhesive System, as well as its transdentinal cytotoxicity and microtensile bond strength to dentin.

Methods: Experimental groups were established according to the time and temperature of the air jet: G1: 5 seconds–25°C; G2: 10 seconds–25°C; G3: 20 seconds–25°C; G4: 5 seconds–50°C; G5: 10 seconds–50°C; G6: 20 seconds–50°C. In control group (G7), no treatment was performed. The DC

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was assessed using the Fourier transform infrared spectroscopy–attenuated total reflectance (FTIR–ATR) technique. For the transdentinal cytotoxicity test, dentin discs fitted in artificial pulp chambers (APC) received the application of the adhesive system and the air jets. For the microtensile bond strength, healthy molars were restored and submitted to the microtensile test after 24 hours and 6 months, respectively.

Results: Significant reduction in viability of Mouse Dental Papilla Cell-23 (MDPC-23), which exhibited morphological changes, was observed in all experimental groups compared to control ($p<0.05$). Although all tested protocols resulted in transdentinal diffusion of 2-hydroxyethyl methacrylate (HEMA), the group G6 presented the highest degree of monomeric conversion and the lowest cytotoxic effect, with higher dentin bond strength values in comparison to group G1 ($p<0.05$).

Conclusions: Applying an air blast at 50°C for 20 seconds increases the DC and microtensile bond strength of the 3M Single Bond Universal Adhesive System to dentin, as well as reduces the transdentinal cytotoxicity of the material to pulp cells.

INTRODUCTION

Universal adhesive systems were developed with the objective of facilitating the adhesive restoration technique. In order to concentrate the multipurpose characteristics of this material into a single vial, a high solvent content was added to new monomeric components, which may diffuse through dentin to reach the pulp tissue.[1-7] Uncured resin monomers in contact with the dental pulp trigger persistent chronic foreign body inflammatory reactions and cause irreversible toxic effects to pulp cells.[8-11]

It is known that during the curing of adhesive systems, there is an incomplete conversion of monomers that remain free in the dentin[12] and that, due to substrate permeability, can diffuse via the dentinal tubules to cause pulp damage.[13,14] Thus, it is essential that the polymerization process is effective, maximizing the physical-mechanical properties, favoring the clinical performance of the product, as well as reducing its possible toxic effect on dental pulp cells.[7]

Due to the fact that the cytotoxicity of resinous materials is directly related to the effectiveness of the polymerization technique,[2,7] some protocols have been tested to increase the degree of conversion (DC) of monomers to polymers. Rising temperature facilitates solvent evaporation, activates free radical mobility, reduces viscosity, and makes polymer chains more flexible, increasing the extent to which monomers can be converted into polymers.[15-20] Thus, by evaluating different heat-curing polymerization methods, some authors have reported improved physical, mechanical, and biological properties of resinous materials.[17,20-28]

As a result, the application of a heat source to the adhesive system may enhance the conversion of monomers to polymers, increasing mechanical properties and reducing material cytotoxicity. Thus, the aim of the present study was to evaluate the influence of heat application on the DC, transdentinal cytotoxicity, and dentin bond strength of a universal adhesive system.

METHODS AND MATERIALS

To perform the experimental tests, the 3M Single Bond Universal Adhesive System was selected (3M Oral Care, 3M Deutschland, Seefeld, Germany; Table 1), based on its previous performance in several studies over time. For the application of the air jet blast, a thermal blower (GuangZhou YiHua Electronic Equipment Co. Ltd., China) was used, modified to promote an air flow at different temperatures, which can vary from room temperature to 70°C, in addition to the use of a tip compatible with the size of a dental cavity.

Degree of Conversion

The degree of conversion (DC) was assessed by a spectrometer (VERTEX 70v, Bruker Optics, Ettlingen, Germany) using the Fourier Transform Infrared Spectroscopy (FTIR) technique equipped with an attenuated full reflectance accessory (ATR)—FTIR–ATR. Aliquots of 5 μL of the adhesive system were applied directly over the diamond crystal to read the material spectrum.[28]

Unpolymerized material was analyzed, followed by specimen preparation (n=3). The specimens were air jet blasted with varying times and temperatures, as shown in Table 2, and photoactivated (VALO, Ultradent Products Inc, Salt Lake City, Utah, USA) for 10 seconds. The percentage of unreacted carbon–carbon double bonds was determined by the ratio of the absorbance intensities between the aliphatic (peak at 1636/cm) and aromatic carbon–carbon (peak at 1608/cm) double bonds at a resolution of 4/cm and 32 scans.

Transdentinal Cytotoxicity

A total of 106 healthy human molars were obtained from the Tooth Bank of the Lutheran University of Brazil, after ethical approval. Using a metallographic

Table 1: *Composition of the 3M Single Bond Universal Adhesive System (3M Oral Care)*	
Adhesive System	**Composition**
Single Bond Universal	Bisphenol Diglycidyl ether dimethacrylate (BisGMA), 2-hydroxyethyl methacrylate (HEMA), silica treated silica ethyl alcohol, decamethylene dimethacrylate, water, 1-10 decanediol phosphate methacrylate, acrylic and itaconic acid copolymer, camphorquinone, N, N -dimethylbenzocaine, 2-dimethylaminoethyl methacrylate, methyl ethyl ketone

cutter (IsoMet 1000, Buehler Ltd, Lake Bluff, IL, USA) equipped with a diamond blade (11-4254, 4"x0.012"/15LC series, Diamond Wafering blade, Buehler Ltda), 0.4 mm dentin disks were obtained from 70 teeth. These discs had their hydraulic permeability determined as previously described by Leite and others.[5] The diameter of the discs was reduced to 8 mm with a high speed cylindrical diamond tip (Diamond Tip FG 3098—KG Sorensen). The discs were then adapted in artificial pulp chambers (APC), and the disc–APC sets were subjected to ethylene oxide sterilization.[5]

Immortalized cells of Mouse Dental Papilla Cell-23 (MDPC-23) odontoblastic lineage stored in liquid nitrogen at the Laboratory of Experimental Pathology and Biomaterials at the Dental School-São Paulo State University (FOAr/UNESP) were thawed and cultured in 100 cm^2 plates (Costar Corp, Cambridge, MA, USA) in Dulbeccos's Modified Eagle Medium culture medium (DMEM; GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, Cultilab, Campinas, SP, Brazil), 100 IU/mL and 100 μg/mL of penicillin and streptomycin, respectively (GIBCO, Grand Island, NY, USA). These cells were subcultured and kept in an incubator containing 5% CO_2 at 37°C until they reached sufficient number to perform the experiment.

Sterile disc–APC assemblies were individually inserted into 24-compartment plates so that the pulp surface of the dentin discs faced upwards. On this surface, 1×10^5 cells were seeded in 20 μL of complete DMEM. After 30 minutes in an incubator (sufficient time for initial adhesion of cells to the dentin substrate), 1 mL of complete DMEM was applied to each compartment of the plates, which were kept in an incubator for an additional 48 hours. At the end of this period, the culture medium was replaced with 1 mL FBS-free DMEM and the disc–APC assemblies inverted in the compartments such that cells adhered to the pulp surface of the disc were kept down and in contact with the DMEM. Thus, the occlusal surface of the dentin discs, now facing upwards, remained exposed to receive the treatments proposed in the present study (Table 2).

Table 2: *Relationship Between Groups and Protocols of Air Jet Application*	
Groups	**Protocols**
G1 [a]	5 seconds of air jet at 25°C
G2	10 seconds of air jet at 25°C
G3	20 seconds of air jet at 25°C
G4	5 seconds of air jet at 50°C
G5	10 seconds of air jet at 50°C
G6	20 seconds of air jet at 50°C
[a]*Protocol recommended by the manufacturer.*	

To perform the adhesive protocols, the occlusal surface of each dentin disc was washed with 1 mL of Phosphate-buffered saline (PBS) with concomitant aspiration, and the excess moisture was removed with sterile absorbent paper. Next, the total volume of 10 μL of the adhesive system was applied for 20 seconds over all dentin-exposed surfaces followed by gentle air jet blast application (Table 2) and photoactivated for 10 seconds with high power LED (VALO, Ultradent), with a light intensity of 1000 mW/cm^2. For this cytotoxicity test, a negative control group (G7) was established where no treatment was performed on the occlusal surface of the dentin discs. Next, the disc–APC sets were incubated in a 5% CO_2 atmosphere at 37°C for 24 hours.

To perform the cell viability test (n=8), the dentin discs were carefully removed from the APCs and individually positioned with the pulp surface containing the cells facing upwards at the bottom of the compartments of new 24-compartment acrylic plates. Next, the culture medium was aspirated with 90 μl DMEM and 10 μl MTT solution (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) (Sigma Chemical Company, St Louis, MO, USA) at a concentration of 5 mg/mL and was applied to each dentin disc. The samples remained for 4 hours in an incubator for formazan crystal formation. A solution with 100 μL of acidified isopropanol was then applied to the discs to dissolve these crystals. After dissolution, 100 μL aliquots were transferred to a 96-well plate (Costar Cat 3595—

Corning Inc, NY, USA) and the medium blue violet stain was quantified on a spectrometer (Synergy H1, BioTek, Winooski, USA) at a wavelength of 570 nm.

To evaluate the cell morphology, dentin discs (n=2) were removed from the APCs, and cells adhered to their surface were fixed with 2.5% glutaraldehyde, washed with PBS, and post-fixed with osmium tetroxide.[5] After being dehydrated in growing ethanol solutions (30%, 50%, 70%, 95%, and 100%), the specimens were chemically dried in 1,1,1,3,3,3- hexamethyldisilazane solution (HMDS, ACROS Organics, New Jersey, USA), mounted on metal stubs, and kept in a desiccator for 72 hours. The pulp surface of the dentin discs containing the cells was covered with gold and analyzed by scanning electron microscopy (JSM-6610; JEOL Ltd, Akishima, Tokyo, Japan).

To quantify the 2-hydroxyethyl methacrylate (HEMA) (n=6), 200 μL aliquots of the extracts (culture medium + dentin-diffused adhesive system components) representative of each group were collected and immediately applied to 96-well plate compartments with specific UV treatment (Corning Costar, New York, USA), and analyzed at an absorbance peak of 231 nm. HEMA concentration was determined by comparative method using a 6-point standard curve with 1:1 serial dilution (10 mM, 5 mM, 2.5 mM, 1.25 mM, 0.625 mM, and 0.312 mM), adapted by a previous study.[28] The mean of the negative control group was used as a blank.

Microtensile Bond Strength

Of the 106 healthy molars selected for the present study, 36 were transversely cut in the occlusal-third of the crown with the aid of a metallographic cutter (IsoMet 1000, Buehler Ltd) equipped with a diamond disk. Next, the sectioned surfaces of the teeth were worn with 600-grit sandpaper mounted on Politriz (ERIOS-27000, Euros, São Paulo, SP, Brazil). This procedure was performed under constant water cooling until a regular and homogeneous dentin surface was obtained, which was inspected with the aid of a stereoscopic magnifying glass (Model SZX7, Olympus, São Paulo, SP, Brazil).

To provide a homogeneous smear layer, the previously cut teeth had their dentin surfaces sanded for 30 seconds with 320-grit sandpaper (T469-Norton, Saint-Gobain Abrasivos Ltda, Jundiaí SP, Brazil). The dentin surface was then washed with distilled water, and the excess moisture was removed with absorbent paper, leaving the surface slightly damp. Next, 20 μL of the adhesive system was applied to the exposed dentin surface. After 20 seconds, solvent evaporation and material photoactivation were performed as described above. The teeth were restored with Filtek Z350 XT composite resin, color A 3.5 (3M from Brazil Ltda, Sumaré, SP, Brazil) using an incremental technique (three increments of 1 mm each, photoactivated for 20 seconds). After restoration, the teeth were stored in deionized water at 37°C for 24 hours.

With the aid of a metallographic cutter (IsoMet 1000, Buehler) equipped with a diamond disc, it was possible to obtain a total of eight sticks from each tooth (0.9×0.9 mm cross-sectional area). Half of the sticks were submitted to the microtensile bond strength test within 24 hours after the restorative procedure. The remaining sticks were immersed in saliva solution (KCl 12.92 mM, KSCN 1.95 mM, $Na_2SO_4 \bullet 10H_2O$ 2.37 mM, HEPES 5 mM, NH_4Cl 3.33 mM, $CaCl_2 \bullet 2H_2O$ 1.55 mM, $NaHCO_3$ 7.51 mM, $ZnCl_2$ 0.02 mM, pH 7.4) and then stored in an incubator at 37°C for 6 months, where they were submitted to the same mechanical test. During the storage period, the saliva solution was renewed monthly.

The specimens were fixed in metal devices of a universal mechanical testing machine (DL 1000, EMIC Testing Systems, São José dos Pinhais, PR, Brazil) using a cyanocrylate adhesive (Super Bonder Gel and Activator 7456, Henkel Loctite Ltda, São Paulo, SP, Brazil). Traction movements were initiated by a specific computer program (Test Works, Star IV, MTS System Corporation, Eden Prairie, MN, USA) and were terminated at the time the specimen ruptured with maximum load values recorded by the program. The fractured specimens were analyzed with a stereoscopic magnifying glass (Model SZX7, Olympus) having a 30-fold increase, and fractures were classified as resin or dentin cohesive, adhesive, or mixed.[30]

Statistical Analysis

Data on the DC, bond strength, and quantification of HEMA diffusion were evaluated by the two-way ANOVA test, followed by the Tukey test for DC and microtensile bond strength. For each period of analysis, the mean of the values of four sticks of each tooth was considered as an experimental unit (n=6). For cell viability, the results were submitted to the one-way ANOVA test, followed by the Tukey test for cell viability. All statistical tests were considered at a significance level of 5%.

RESULTS

Degree of Conversion

The highest monomeric conversion values were observed in the group where the adhesive system was heated by applying a 50°C air jet for 20 seconds, as shown in Table 3.

Table 3: *Average of the Degree of Conversion (DC) Values (%) According to Air Jet Temperature and Time*[a]

Time	Temperature	
	25°C	50°C
5s	55.8 ± 1.7 Aa (G1)	53.4 ± 1.5 Ca (G4)
10s	56.7 ± 0.7 Aa (G2)	60.4 ± 1.9 Bb (G5)
20s	57.3 ± 1.6 Aa (G3)	66.4 ± 3.1 Ab (G6)

[a]*Lowercase letters indicate difference between temperatures (same line); uppercase letters indicate time difference (same column).*

Transdentinal Cytotoxicity

Cell Viability—

For all groups, the adhesive systems caused a high cytotoxicity index when compared to the negative control group ($p<0.05$), as observed in Figure 1. However, in the group where the adhesive system received application of 50°C air jet blast for 20 seconds, the cells showed significantly higher viability than those belonging to the group where the material was blown at 25°C for 5 seconds ($p<0.05$).

Cell Morphology—

As shown in Figure 2, MDPC-23 pulp cytotoxicity occurred in all experimental groups, when compared to the negative control. This toxic action resulting from the treatments was determined by the reduction in the number of cells that remained attached to the pulp surface of the dentin discs, as well as by the morphological alteration of these cells, which had reduced size associated or not with the rupture of the cytoplasmic membrane. The most intense negative effects on cells were observed in group G1, where the adhesive system was blown at 25°C for 5 seconds.

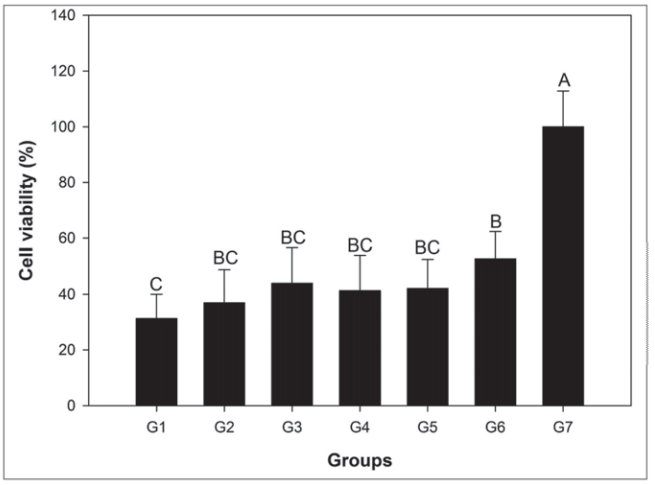

Figure 1. *Mean and standard deviation of cell viability values (%) of experimental and control groups. Different letters show significant difference between groups (*p*<0.05).*

HEMA Diffusion Quantification—

Although the amount of HEMA diffused through the dentin was not statistically different when the experimental groups were compared with each other ($p>0.05$), it was possible to observe a tendency of reduction in HEMA diffusion values with increasing the time and air jet temperature applied over the adhesive system (Figure 3).

Microtensile Bond Strength

The failure mode distribution of the specimens in both the periods of analysis is shown in Table 4. In general, it was observed that the failures occurred predominantly at the adhesive interface.

As shown in Figure 4, the increased air jet application time resulted in an increase in dentin bond strength, both in the 24-hour and 6-month analyses. Similarly, the use of heat over the same period of time resulted in better dentin bond strength results. The increase in air jet time and temperature provided an increase in bond strength.

It is noteworthy that the application protocol of the dentin adhesive system recommended by the manufacturer presented the least favorable results of bond strength in the 24-hour analysis, and which were even worse in the 6-month period. Differently, the application of an air jet at 50°C for 20 seconds presented the best results in the analysis after 6 months.

DISCUSSION

Increasing temperature provides greater mobility of the photoinitiators and monomers present in the resin matrix, as well as an increase in molecular kinetic energy, facilitating breakage of intermolecular bonds between solvents and resin monomers.[17,31] Consequently, the combination of higher solvent evaporation and increased vibrational energy of molecules favors

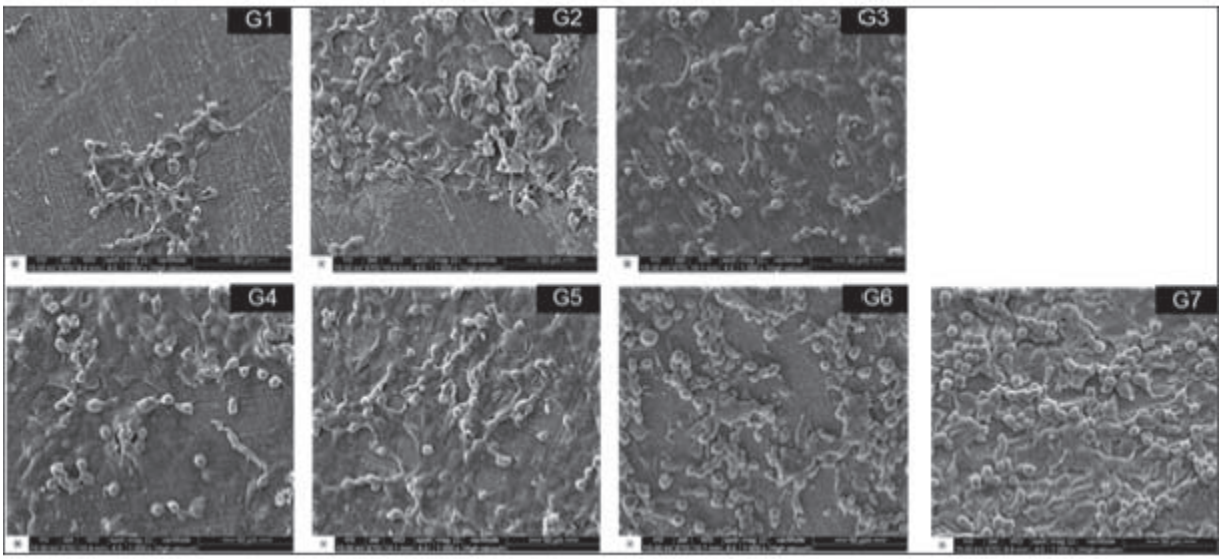

Figure 2. *SEM, 1000× Representative images of Mouse Dental Papilla Cell-23 (MDPC-23) cells sown on the surface and pulp of the dentin discs of the experimental and control groups. G1–G6: The application of the adhesive system on the occlusal surface of the dentin discs caused intense cytotoxicity response into the MDPC-23 odontoblast cells shown on the pulp surface, promoting morphological changes such as size reduction and loss of cytoplasmic processes. The most intense degree of cytotoxicity was presented in G1, where a large number of damaged cells detached from the dentin disc. G7 (control): MDPC-23 cells displaying an intact cytoplasmic membrane covering virtually all dentinal substrate.*

monomeric conversion,[20] reducing the amount of free monomers with potential to diffuse through the dentin to cause damage to the pulp cells.[1-3,5]

In the present study, it was possible to identify a higher degree of monomeric conversion by increasing the time and temperature of the air jet applied over the adhesive system. Previous studies used temperatures from 37°C to 60°C to promote greater solvent evaporation and increase the DC.[20-22,31-35] Silva and others[36] demonstrated that applying 60°C air jet blast on 0.5 mm thick dentin for 10, 20, 30, and 40 seconds increased the pulp temperature by 5.8°C, 10.1°C, 13.6°C, and 16.6°C, respectively. These thermal variations may possibly cause pulp damage, since it has been reported that an increase between 5.5°C and 11°C in the intrapulp temperature results in different levels of primate pulp necrosis.[37] Under other conditions, Baldissara and others[38] demonstrated that a 9-15°C increase in intrapulp temperature was not sufficient to cause pulp necrosis after 3 months.

In the present research, two temperatures (25°C and 50°C) and three application times of air jet for solvent

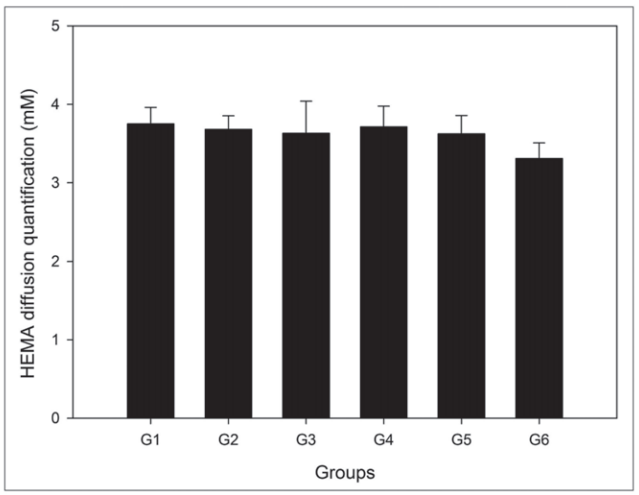

Figure 3. *Mean and standard deviation of 2-hydroxyethyl methacrylate (HEMA) diffusion quantification values (mM). There was no statistical difference between groups.*

Table 4: *Types of Fractures Presented During μTBS 24 h and 6 Months*

Groups	Types of Fractures							
	24 h				6 m			
	A	M	D	R	A	M	D	R
G1	19	1	3	1	15	2	3	4
G2	16	2	1	5	13	4	3	4
G3	14	2	1	7	15	0	7	2
G4	18	1	2	3	15	0	1	8
G5	16	0	6	2	13	3	3	5
G6	14	3	3	4	13	2	1	8

Abbreviations: A, adhesive; D, dentin cohesive; M, mixed; R, resin cohesive.

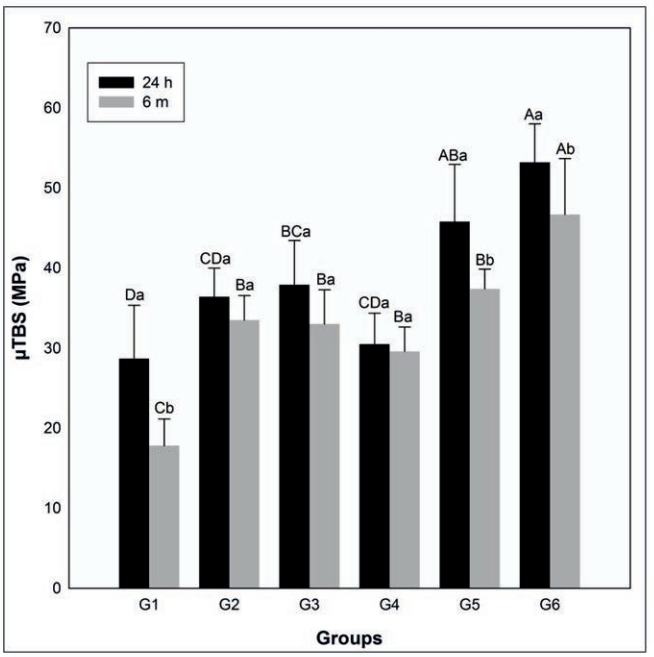

Figure 4. *Mean and standard deviation of the bond strength values in the immediate and late analyses (n=6; ANOVA test; α =0.05). Different upper case letters show significant difference between the groups in the same analysis period, while different lower case letters indicate significant difference of each group in the different analysis periods.*

evaporation of the adhesive system (5, 10, and 20 seconds) were evaluated. In all experimental groups, there was a significant reduction in cell viability compared to the control group (G7). Overall, the cell damage observed in the present study was not only limited to reduced mitochondrial activity but was also characterized by morphological changes in pulp cells, which exhibited cytoskeleton contraction and loss or shortening of cytoplasmic prolongation. In these experimental groups, a reduction in the number of cells adhered to the dentin substrate was also observed, indicating the occurrence of cell death. However, it was determined that the smallest damage occurred when a 50°C air jet blast was applied for 20 seconds over the occlusal surface of the dentin discs. Thus, in addition to increasing the degree of monomer conversion,[20] the air jet blast at a temperature of 50°C was the least aggressive to the pulp cells. This positive result regarding cell number maintenance and viability may have been due to the fact that fewer free monomers were able to diffuse through the dentin to cause toxic effects on the pulp cells. Previous studies have shown that transdentinal toxicity of adhesive systems is directly related to the amount of free monomers released from the material that can diffuse through the dentinal tubules.[1,39] Thus, higher cytotoxicity was observed in the group where the 25°C air jet blast was directed for 5 seconds (manufacturer's recommendation) on the adhesive system applied to the occlusal surface of the dentin disc. The scientific data obtained in the present research supports the idea that the higher the degree of monomer conversion the lower the cytotoxicity of resinous materials, as recently reported by Fujioka-Kobayashi and others.[40]

Although resinous materials have different types of monomers in their composition, which can cause toxic effects of varying intensity on pulp cells, HEMA is the major component of adhesive systems responsible for transdentinal toxicity.[6] Although no statistical difference was observed, there was a tendency for a lower transdentinal diffusion of HEMA with increasing time and temperature of the air jet applied over the adhesive system. Perduns and others[41] reported that slight variations in the amount of HEMA may influence the level of cytotoxicity of this resinous monomer on pulp cells. The authors demonstrated that 0.5 mM HEMA is sufficient to induce the expression of genes related to cellular oxidative stress; higher concentrations of this monomer result in overload to the antioxidant system, which causes cell death and modulates inflammatory and metabolic pathways that modify the extracellular matrix.

In a previous study, where the authors also used the 0.4 mm thick dentin discs model mounted on APCs, it was shown that the 3M Single Bond Universal Adhesive System, used in the conventional technique or as self-etching (wet or dry dentin), reduced cell viability by about 88%.[5] In the present study, by applying the same adhesive system as a self-etching agent on the wet dentin substrate, varying the time and temperature of the air jet for solvent evaporation, a reduction in cell viability was observed between 47.3% and 68.7%. It is possible to suggest that the higher transdentinal toxicity observed in the study by Leite and others[5] occurred due to the fact that the authors actively applied the adhesive system on the dentin, which did not happen in the present study. The active application technique is recommended by the manufacturer to favor the infiltration of resin monomers into demineralized dentin, which seems to result in the formation of a more homogeneous hybrid layer.[22] However, it is known that the active application of the adhesive on the dentin may also increase the transdentinal diffusion of free monomers to damage pulp cells.[42]

The use of standardized laboratory protocols, especially those employing standardized permeate dentin barriers to assess the indirect cytotoxicity of dental materials, is necessary to ensure the reliability and reproducibility of studies.[14,43] The use of dentin

barriers in *in vitro* research approximates laboratory protocols to clinical conditions and helps to understand how dentin characteristics may affect the diffusion of molecules from experimental materials at concentrations that may cause pulp cell toxicity.[1,14] Due to the need to assess the biocompatibility of dental materials and new clinical procedures, cell culture studies should be adopted as a prior approach to *in vivo* evaluations and clinical trials.[43] Even with all the advantages of *in vitro* studies, it is known that the results of these laboratory studies should not be directly extrapolated to clinical situations.[14] Thus, the authors of the present study recognize the limitation of the data obtained and the need for future-controlled clinical investigations, in order to determine the safe application of the procedures successfully tested here.

To evaluate the mechanical properties involved in the formation of the hybrid layer, the bond strength to dentin was evaluated. Klein-Junior and others[21] observed higher resin–dentin bond strength by applying an air jet blast at 60°C for 10 seconds, while Fu and others[44] and Saikaew and others[45] obtained better results by increasing the air jet application time. Rising temperatures are known to increase the kinetic energy of molecules, altering the way they bond together, favoring polymerization.[20] On the other hand, prolonging the air jet blast application time promotes greater solvent evaporation, resulting in a more efficient polymerization, which may favor the bond strength of the material with the dental substrate.[44,45] In the present study, the times of 5, 10, and 20 seconds of application of the air jets were tested, which associated with the increase of the temperature generally resulting in an increase of bond strength. The use of air jet blast, as recommended by the manufacturer (5 seconds), caused a reduction in bond strength, which was lower than in the other groups within 6 months of analysis. However, the application of 20 seconds of 50°C air jet resulted in increased bond strength, which was superior to the other groups evaluated within 6 months.

Finally, given the limitations of the present *in vitro* study, it is possible to suggest that the increase in temperature and time of application of the air jet over the 3M Single Bond Universal Adhesive System favors the DC and bond strength of the material to dentin, as well as decreasing its transdentinal cytotoxicity. In the present laboratory study, we simulated a very deep cavity through the use of 0.4 mm thick dentin discs. This protocol aimed to expose the adhesive system and the solvent evaporation techniques under test to a maximum toxicity challenge. However, regardless of the techniques evaluated in the present study, it is known that the application of adhesive systems in very deep cavities is still contraindicated, since these resinous materials, used as self-etching agents or on acid-etched dentin, cause intense pulp toxicity.[9,10,14] Therefore, the use of lining agents with important mechanical and biological properties has been recommended prior to the application of the adhesive system in cavities whose walls are very close to the pulp.[46]

CONCLUSION

According to the methodology employed in the present *in vitro* study, it was concluded that the application of air jet blast at a temperature of 50°C for 20 seconds favored the monomeric conversion of the 3M Single Bond Universal Adhesive System, reducing the transdentinal toxicity of this adhesive on the pulp cells as well as increased bond strength of the material to dentin.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of Lutheran University of Brazil. The approval code issued for this study is 89363218.3.0000.5349.

Conflict of Interest

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

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Mechanical Properties of Bisacryl-, Composite-, and Ceramic-resin Restorative Materials

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Clinical Relevance

Understanding mechanical properties and wear resistance of resin-based materials aids in material selection and enhances clinical performance. Certain bisacryl resin materials may have favorable mechanical properties and resistance to wear to suggest a longer term of use than commonly intended.

SUMMARY

Objective: Resin-based materials used in restorative dentistry are introduced at a fast pace with limited knowledge about their properties. Comparing properties of these materials from different restorative categories is lacking but can help the clinician in material selection. This study aimed to compare mechanical properties and wear resistance of bis-acryl-, composite-, and ceramic-resin restorative materials.

Methods and Materials: Bisacryl-resin (Bis-R, LuxaCrown, DMG), composite-resin (Com-R, Filtek Supreme Ultra, 3M Oral Care), and ceramic-resin (Cer-R, Enamic, VITA Zahnfabrik) specimens were prepared for mechanical tests: fracture toughness (FT) with and without initial thermomechanical loading using a mastication simulator, flexural strength (FS), and flexural modulus (FM), compressive strength (CS), and volumetric wear loss measurement. The datasets for FT and wear resistance were each analyzed using two-way ANOVA followed by pairwise comparisons or Tukey testing as appropriate. The datasets for FS, FM, and CS were analyzed using one-way ANOVA followed by the Tukey test.

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Results: Analysis of FS, FM, and CS showed significant differences between materials, with all pairwise comparisons between materials showing significance. Analysis of FT resulted in a significant interaction between the material and treatment, with analysis of wear loss showing a significant interaction between the material and the number of cycles.

Conclusions: Cer-R demonstrated superior FT, CS, and wear resistance compared to Bis-R and Comp-R materials. Fracture toughness of Bis-R increased after thermomechanical loading.

INTRODUCTION

Resin-based materials have a wide range of use in modern dentistry and have become a popular alternative to traditional ceramic and metal restorations.[1,2] Nowadays, resin-based materials encompass a wide variety of dental materials including provisional materials, conventional composite-resin, and CAD/CAM blocks. However, there are still issues surrounding resin-based restorations such as relatively poor mechanical properties and wear resistance when compared to ceramic material.[3,4]

Provisional materials are used to protect and maintain remaining tooth structure and function during the fabrication of a permanent prosthesis. It is important that the provisional material has acceptable biological, mechanical, and physical properties to ensure the protection of hard and soft tissues throughout treatment.[5] Although provisional materials are meant to be replaced by permanent prostheses, they may need to survive for periods greater than six months depending on the treatment plan.[3] Since its emergence in the late 1990's, bisacryl has become a popular choice for provisional restorations. Bisacryl resin-based materials offer superior mechanical properties and wear resistance compared to earlier counterparts.[6] A variety of polymerization methods also make bisacryl a popular choice for clinicians. However, it has been reported that dual-polymerizing materials may show inferior flexural strength if they are only allowed to polymerize chemically.[5]

Composite-resin is a popular choice for direct restorations due to its ease of handling, natural esthetics, and relatively strong mechanical properties. Although composite-resins lack the strength of ceramics, their hardness and flexural properties are similar to natural teeth, which help limit opposing wear.[7,8] The composition of the material is a primary factor in determining the mechanical properties. For example, Bis-GMA has hydrophilic properties, which may affect the mechanical strength of the material through increased water sorption and surface degradation.[3] The quantity and size of the inorganic filler can also influence the overall mechanical strength and wear pattern of the material.[9]

CAD/CAM technology was first developed with CEREC in 1985, giving the ability to produce in-office restorations through milling.[10] This technological breakthrough provided the ability to reproduce consistent esthetic restorations in a time-efficient manner that often require minor additional processing.[4] The two main CAD/CAM groups consisted of ceramic and composite, each having issues with longevity due to the brittleness of ceramic and the wear resistance and poor mechanical strength of composite. The introduction of a polymer-infused ceramic CAD/CAM material attempted to address this issue by combining the positive properties of both ceramic and composite.[4] The polymer network provides increased flexural strength and minimized opposing wear, while the ceramic matrix provides improved wear resistance and strength.[11] As suggested by He and Swain, this combination provides similar characteristics to natural teeth, thus making it an ideal restorative material.[8]

A review of the literature indicates that a comparison between bisacryl-, composite-, and ceramic-resin materials focusing on material selection for clinicians has not been performed. As a result, comprehensive analysis of mechanical properties and wear resistance of these materials is lacking. Therefore, the aim of this study was to provide a detailed comparison of mechanical properties and wear resistance of resin-based materials. The two null hypotheses were: 1) There is no difference in fracture toughness values between the different types of resin-based materials, and 2) thermomechanical loading has no effect on the fracture toughness values of the investigated materials. There is no difference in flexural strength, flexural modulus, and compressive strength properties of the resin-based materials. There is no difference in the wear resistance of the resin-based materials investigated.

METHODS AND MATERIALS

Bisacryl-resin (Bis-R, LuxaCrown [LC], DMG Chemisch-Pharmazeutische, Hamburg, Germany), composite-resin (Com-R, Filtek Supreme Ultra, 3M Oral Care, St. Paul, MN, USA), and ceramic-resin (Cer-R, Enamic, VITA Zahnfabrik, Bad Säckingen, Germany) CAD/CAM blocks (Table 1) were prepared for the following mechanical tests:

- Fracture toughness (FT); with and without initial thermomechanical loading using a mastication simulator.

Table 1: *Resin-based Materials Studied*

Material	Manufacturer	Shade/Lot Number(s)	Code
LuxaCrown	DMG	A2/789645	Bis-R
Filtek Supreme Ultra	3M Oral Care	A2B/N967677	Com-R
Enamic	VITA	2M2/78140	Cer-R

- Flexural strength, and flexural modulus (FS, FM).
- Compressive strength (CS).
- Volumetric wear loss measurement.

Fracture Toughness

The FT of the studied materials were measured using the single edge V-notched beam under a three- point bending test. The preparation and testing parameters followed ASTMD5045-14; ISO/NP 13586.[12,13]

A custom mold was created (21.0 ± 0.1 mm in length, 4.0 ± 0.1 mm in depth, and 3.0 ± 0.1 mm in thickness) from polyvinylsiloxane (PVS) impression material for specimen preparation. Materials were carefully injected into the mold and covered by a transparent ethylene film and glass slide. Slight pressure (5-10 N) was applied to the center of the glass slide to evenly distribute the material and extrude excess. Bis-R specimens were allowed to self-polymerize according to the manufacturer's recommended time. Com-R specimens were carefully photo-polymerized according to the manufacturer's recommended time of exposure using a visible photo-polymerizing unit (Elipar DeepCure-S, 3M Oral Care) with mean irradiance of 1200 mW/cm^2. The irradiance of the photo-polymerizing unit was tested every 24 hours using the MARC Light Collector (BlueLight Analytics, Halifax, NS, Canada) to ensure the consistency of polymerizing conditions. Each specimen was polymerized in three + overlapping irradiations to ensure efficient polymerization of the specimen. Each specimen was inspected for defects prior to polishing. If defects were significant, they were discarded. Remaining specimens were polished under water using 600-grit silicon-carbide abrasive paper (MicroCut, Buehler, Lake Bluff, IL, USA) to remove excess material. A digital micrometer with an accuracy of 0.01 mm, (QuantuMike Micrometer, Mitutoyo Corporation, Sakado, Japan) was used to monitor the dimensions during polishing. The final width (b) and thickness (w) of each specimen was recorded before storing in deionized water at 37°C for 24 hours prior to testing.

Cer-R blocks were sectioned into smaller workable blocks to be further sectioned using an IsoMet-1000 sectioning saw. A 15 HC diamond coated blade (Buehler) was wafered under water at 150 rpm according to manufacturer's recommendations. The Cer-R block was fixed to a flat vice and secured by melted wax during sectioning. To achieve the final specimen dimension, three consecutive cuts were made (21.0 ± 0.1 mm in length, 4.0 ± 0.1 mm in depth, and 3.0 ± 0.1 mm in thickness). Due to the accuracy of the sectioning machine, no further processing on Cer-R was necessary prior to testing. Specimens were stored in a dry, air-tight container until testing.

Specimens were remounted in the IsoMet 1000 to create a 0.50-mm deep notch at the center using a 150-μm thick diamond coated blade. The notch was then coated with diamond polishing paste (3.5 μm, Kent Supplies, Quebec, Canada) and a razor blade was used to form the notch into a V-shape with a final depth of 0.80 mm to 1.20 mm. A consistent horizontal motion and force (5 N-10 N) with the razor blade ensured a uniform notch formation. Each side of the notch was measured using a light microscope with a >50× magnification and averaged for a final notch depth.

A Universal Instron machine (Model 4411, Instron, Norwood, MA, USA) with an attached 3-point bending fixture was used to determine the FT of the specimens. Specimens were placed evenly on the fixture and loaded until failure with a crosshead speed of 0.5 mm/min. The peak fracture load was recorded to three significant figures and the FT was determined in units of MPa$\cdot$m$^{1/2}$ according to the formula:

$$K_{IC} = (P / bw^{1/2}) * (L / w) * ((3\,\alpha^{1/2}) / ((2\,(1-\alpha))^{3/2})) * Y,$$

where $Y = 1.9887 - (1.326*\alpha) - (3.49-0.68*\alpha) + (1.35\alpha^2)(\alpha)(1-\alpha)/(1+\alpha^2)$, α = average V-notch depth of the group, P = fracture load, b = width of the specimen, w = thickness of the specimen, and L = distance between support beams.

Thermomechanical Loading

Specimens for FT were separately prepared for thermomechanical loading. Each group was mounted in a custom fabricated stainless-steel holder with acrylic resin. The acrylic resin was then allowed to fully set following the manufacturer's recommended time prior

to loading. Stainless steel holders were then mounted in the masticating simulator (CS-4.8, SD Mechatronik, Feldkirchen-Westerham, Germany). The force was calibrated using a force meter (KM-3, SD Mechatronik) with a weight of 4 kg mounted to an antagonist bar (1 kg). The machine was set to 100 cycles to obtain an average z-axis force. The testing parameters were set for 1,200,000 mechanical cycles (1.2 Hz) at 50 N with simultaneous thermocycling in deionized water (5° and 55°C) for a 30-second dwell time.[14] A break detection system (PM-3, SD Mechatronik) was installed in each chamber and monitored any premature fractures. Surviving specimens were tested for FT.

Flexural Strength and Modulus (FS and FM)

The FS (MPa) and FM (GPa) was determined using a three-point bending test. The testing parameters and preparation followed ISO Standard 4049.[15]

A custom mold (21.0 ± 0.1 mm in length, 2.0 ± 0.1 mm in depth and 2.0 ± 0.1 mm in thickness) was fabricated from PVS for specimen preparation. Bis-R and Com-R were polymerized, finished, and stored for testing as previously described.

Cer-R CAD/CAM blocks were sectioned into smaller workable blocks as previously described. To achieve the final specimen dimension, three consecutive cuts were made (21.0 ± 0.1 mm in length, 2.0 ± 0.1 mm in depth, and 2.0 ± 0.1 mm in thickness). Specimens were stored as previously described.

A Universal Instron machine with an attached three-point bending fixture was used to determine the FS and FM of the specimens. Prior to loading, specimen dimensions were imputed to determine modulus. Specimens were placed evenly on the fixture and loaded until failure with a crosshead speed of 0.5 mm/min. The peak fracture load and modulus were recorded to three significant figures and the FS was determined according to the formula:

$$\alpha = 3\,FL/2wt,$$

where F = maximum force applied, L = distance between support beams, w = width of specimen, and t = thickness of specimen.

Compressive Strength

The CS of the studied materials was determined and analyzed according to ISO Standard 9917-1. A custom mold (6.0 ± 0.1 mm in length and 4 ± 0.1 mm in diameter) was fabricated from PVS for specimen preparation. Bis-R and Com-R were polymerized as previously described.

Bis-R and Com-R were polished under water using 600-grit silicon-carbide abrasive paper (MicroCut) to achieve the desired specimen height. A digital micrometer was used to confirm the length and diameter of each specimen. The diameter was measured twice, each at 90° from the previous and averaged. Specimens were stored for testing as previously described.

Cer-R CAD/CAM blocks were used to create cylindrical specimens using a milling machine. Blocks were mounted in the machine and computer-generated models of the specimens were created. After milling, the dimensions were confirmed and the specimens stored according to the methodologies previously described.

A Universal Instron machine was used to calculate the peak of each specimen. Calibration of the Instron was done prior to testing according to manufacturer's instructions. Cylindrical-shaped specimens were placed flat at the center of the compression plate and loaded until fracture with a crosshead speed of 0.5 mm/min. The peak load was recorded, and the CS was determined according to the formula:

$$\text{Compressive strength} = F/\varpi r^2,$$

where F = maximum force applied and r = radius of the specimen.

Volumetric Wear Loss

The volumetric wear loss of the studied materials was determined using the masticating simulator and the wear measurement system. Eight specimens were prepared for each group (N=24) using custom-made (inner Ø 10 mm, depth 2 mm) stainless steel holders. Bis-R and Com-R were injected into the holders and polymerized as previously described.

Cer-R specimens (n=8) were prepared from CAD/CAM blocks using an IsoMet 1000 (Buehler) sectioning machine. Blocks were mounted to the vise arm and sectioned in 2-mm discs with a diamond-coated blade. Cer-R discs were then mounted in custom made (inner Ø 18 mm, depth 3 mm) stainless steel holders using acrylic resin.

All specimens were polished in a graded series to establish a fine finished surface. Excess material was removed using 600-grit silicon carbide abrasive paper and then finished with 1200-grit silicon carbide paper under running water at 400 rpm for 1 minute per side. Specimens were then stored as previously described.

Each group was mounted in the masticating simulator and the wear measurement system was calibrated to establish a zero-point for each chamber. Steatite balls (Ø-6 mm) were used as antagonists to simulate enamel hardness.

Specimens were submitted to a wear test, measuring the progression of wear after 5k, 10k, 20k, 40k, 60k, 80k, 100k, and 120k cycles.

The following parameters were set in the masticating simulator for thermomechanical loading: Load - 50 N; Upstroke - 2 mm; Downstroke - 1 mm; Horizontal movement - 0.7 mm; Upward speed - 60 mm/second; Downward speed - 60 mm/second; Horizontal speed - 40 mm/second; Frequency - 1 Hz; Thermocycling - 5-55°C 30-second holding time, transfer time 15 seconds, total cycle 90 seconds; Direction - Back and forth.

After each cycle interval was complete, light-body (Honigum Pro Light, DMG America, Ridgefield Park, NJ, USA) and putty (Virtual Putty Fast Set, Ivoclar Vivadent, Amherst, NY, USA) PVS impression materials were used to record the wear. Putty was hand mixed with a 1:1 ratio (base:catalyst) and quickly placed inside of a cylindrical tray before setting. Light-body impression material was then inserted into the wear mark and the tray was placed over the specimen. The light-body impression material was allowed to fully set before removing the tray for inspection. If any defects were present, the impression was retaken. Impressions were scanned using a 3D laser scanner (LAS-20, SD Mechatronik) with a 0.2 mm resolution. The digital scan was then uploaded to Geomagic Control X (3D Systems, Rock Hill, SC, USA) to calculate the volumetric wear loss. Using the digital points uploaded from the scan, a 3D model of the wear mark was created. The volume of the wear was calculated at each measurement interval to create a trend for each material.

Data Analyses

The FT data was summarized by means and 95% confidence intervals for each material and condition. The Akaike information criteria[16] (AIC) and the Bayesian information criteria[17] (BIC) that produced an optimized fit[18,19] of this dataset was determined. Then these data were analyzed by a two-way ANOVA with the interaction term included in the model, using maximum likelihood estimates and a lognormal error distribution (PROC GLMMIX, SAS Proprietary Software 9.4, SAS Institute Inc., Cary, NC, USA). For any effect found statistically significant, the overall effect was analyzed by Bonferroni-corrected (SAS PROC MULTTEST) pairwise comparisons that were *a priori* determined to reflect clinical interest. For each of the FS, FM, and CS properties, the data were summarized by means and 95% confidence intervals for each material. The AIC and BIC that produced an optimized fit of each dataset was determined. Then each of these three data sets were analyzed by a one-way ANOVA, using maximum likelihood estimates and a lognormal error distribution, with any found significant effect resolved further by Tukey testing. The wear data were summarized by means and 95% confidence intervals for each material and number of cycles. The AIC and BIC that produced an optimized fit of this dataset were determined. Then this dataset was analyzed by a two-way repeated-measures ANOVA with the interaction term included in the model, using maximum likelihood estimates, a normal error distribution, the Satterthwaite degrees of freedom method, and a covariance structure of compound symmetry (PROC MIXED, SAS Proprietary Software 9.4). For any effect found statistically significant, the overall effect was analyzed by Bonferroni-corrected pairwise comparisons that were *a priori* determined to reflect clinical interest. Each ANOVA and associated subsequent pairwise comparisons used an overall $\alpha = 0.05$ within each property.

RESULTS

The means and 95% confidence limits of the fracture toughness data are provided in Figure 1. Analysis of the FT data resulted in a significant interaction [$F(2:54)=36.12$, $p<0.0001$)], so pairwise comparisons were made between the two conditions for each material and between all possible pairs of materials for each condition. There were significant differences between the two conditions for each material, with Bis-R and Cer-R each getting tougher on thermomechanical loading ($p\leq0.0013$), and Com-R getting less tough ($p<0.0001$). As formed, Bis-R had lower toughness than either of the other materials ($p<0.0001$) and post-

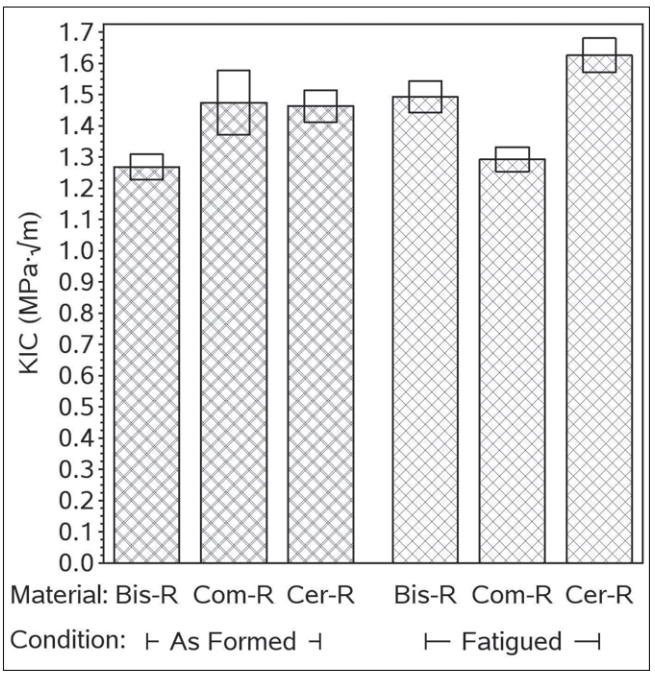

Figure 1. *Means and 95% confidence intervals of fracture toughness for materials and conditions studied.*

thermomechanical loading, and significant differences were found between every pair of materials ($p \leq 0.0144$).

The means and 95% confidence intervals for the FS and FM data are shown in Figure 2. Analysis of the FS data showed a significant difference between materials [$F(2:27)=95.02$, $p<0.0001$] and all pairwise comparisons between materials showed significance ($p \leq 0.0005$). Analysis of the FM data showed a significant difference between materials [$F(2:27)=4587$, $p<0.0001$] and all pairwise comparisons between materials showed significance ($p<0.0001$).

The means and 95% confidence intervals for the CS data are shown in Figure 3. Analysis of the strength data showed a significant difference between materials [$F(2:27)=164.6$, $p<0.0001$], with all pairwise comparisons significant ($p \leq 0.0373$).

The means and 95% confidence intervals for the wear data are shown in Figure 4 for the materials and number of wear cycles studied. Analysis of the wear data showed a significant interaction between materials and the number of cycles [$F(16:168)=11.10$, $p<0.0001$]. Therefore, pairwise comparisons were only evaluated between all possible pairs of materials at each number of cycles and between all possible pairs of number of cycles for each material. For material pair comparisons, the wear of Bis-R was greater than that of Com-R ($p=0.0415$) at 5 k cycles. The wear of Cer-R was greater than that of Com-R at 20 k cycles ($p<0.0001$) and at 40 k cycles ($p=0.0089$). Then at 120 k cycles, the wear of Cer-R was less than that of Com-R ($p<0.0001$) and that of Bis-R ($p=0.0017$). Table 2 indicates statistically significant differences found between numbers of cycles for each material.

DISCUSSION

Resin-based materials are increasingly used in restorative dentistry due to their acceptable strength, wear, elasticity, and affordability when compared to ceramic materials. Therefore, it is important to consider the mechanical characteristics of resin-based materials used in different clinical situations to facilitate their accurate selection favoring clinical longevity. This present laboratory study was performed under controlled conditions to provide a side-by-side comparison of commonly used resin-based restorative materials from different categories known to clinicians as being temporary-, mid-, and long-term restorative material options.

FT is the ability of the material to resist crack propagation and presents a positive correlation with clinical failure; flexural strength presents a positive correlation with wear.[20] FT has also been considered an acceptable method of assessing the mechanical strength and long-term clinical success of a material.[21] Lucsanszky and Ruse found that FT is significantly affected by aging in resin-based materials.[22] In this laboratory study, FT was evaluated pre- and post- thermomechanical loading. Fatiguing may mimic the vertical and lateral occlusal forces in addition to thermal stressing of the material, providing an environment similar, to some extent, to

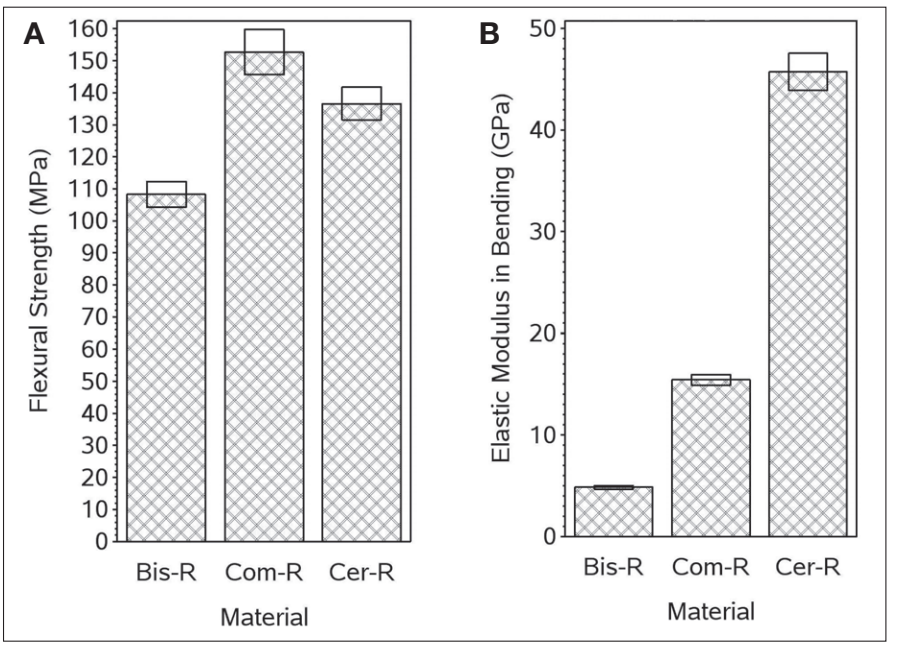

Figure 2. *Means and 95% confidence intervals of flexural strength (a) and of flexural modulus (b) for materials studied.*

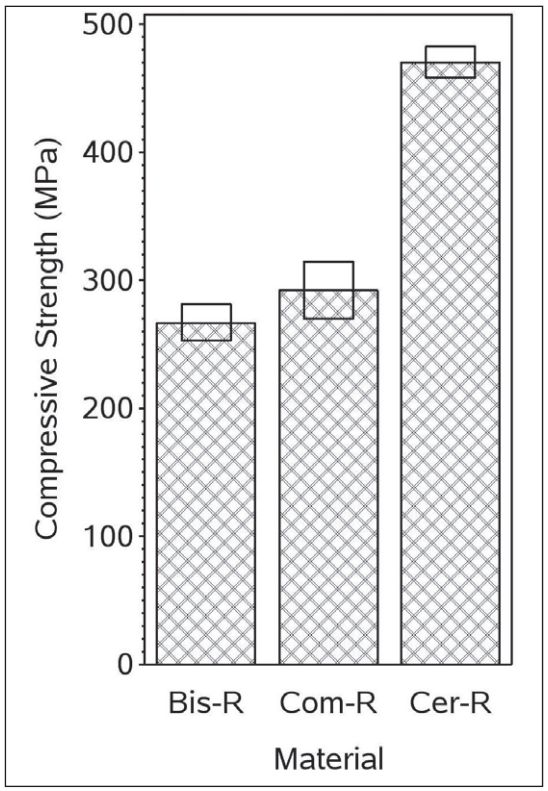

Figure 3. *Means and 95% confidence intervals of compressive strength for materials studied.*

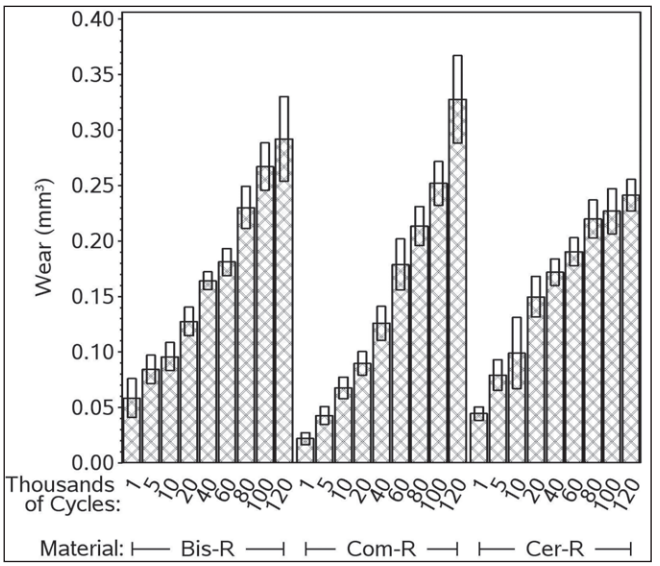

Figure 4. *Means and 95% confidence intervals of wear volume for materials and number of cycles studied.*

the oral environment. In resin-based materials, the matrix and filler play a major role in the mechanical property of the material. The FT of Com-R and Cer-R were greater prefatiguing than Bis-R. The presence of urethane dimethacrylate in Com-R and Cer-R may help contribute to the toughness of the material due to the increased degree of polymerization and flexibility of the urethane linkages.[23] However, Bis-R and Cer-R showed a significant increase in FT after fatiguing while a decrease in the FT of Com-R was observed. The increase in the FT of Bis-R and Cer-R may be attributed to the absorption of water during fatiguing, causing an increase in flexibility, lowering internal stress caused by polymerization shrinkage and increasing the plastic zone. However, the uptake of water in Com-R may have caused swelling and degradation of the matrix and hydrolytic breakdown of the filler-matrix interface, which led to a decrease in mechanical properties.[24] Similar studies reported an increase in the FT of Cer-R after aging, which may be attributed to the increase in flexural modulus.[22,25] Since Cer-R is composed primarily of a ceramic core, it may not have been affected by the absorption of water to the extent of Com-R. The Bis-R material performed favorably after fatiguing, which may be related to the nature of its methacrylate/glass filler composition. Based on these outcomes, the first null hypothesis was rejected. There were differences in FT values of the resin-based materials as formed, and the thermomechanical loading had a significant effect on the FT values of the materials.

Statistical analysis of the results showed Com-R to have the greatest flexural strength of the materials tested, followed by Cer-R and Bis-R. Cer-R lacks the flexibility of Com-R due to the ceramic core, and therefore it is unable to withstand the tension and compressive forces of the three-point bending. Bis-R had a lower FS than the other resin materials and this was supported by its low FM, which was significantly lower as well. Therefore, with the differences in FS and FM of the different materials, the second null hypothesis was rejected.

CS is another acceptable method of evaluating the overall strength of a material. One of the main causes of failure in posterior restorations is caused by compressive forces, therefore it is important for a restorative material to have a CS capable of resisting the forces produced in the mouth.[26]

Cer-R withstood the greatest compressive force of the materials tested, followed by Com- R and Bis-R. High CS of Cer-R may be expected due to the strength of the ceramic core and added flexibility provided by the infiltrated resin.[27] Bis-R and Com-R lack the strength of a ceramic, and therefore are unable to withstand high compressive force. However, it is commonly known that bisacryl-resins are lower in strength than composite resins and hence their temporary restorative

Table 2: *Significant Difference in Wear Between Number of Cycles for Each Material Studied*

Material	Number of Cycles		*p*-value[b]
	Lower	Least of Upper[a]	
Cer-R	1000	10,000	<.0001
Com-R	1000	10,000	0.0007
Bis-R	1000	10,000	0.0223
Cer-R	5000	20,000	<0.0001
Com-R	5000	20,000	0.0004
Bis-R	5000	20,000	0.0021
Cer-R	10,000	20,000	0.0001
Com-R	10,000	40,000	<0.0001
Bis-R	10,000	40,000	<0.0001
Cer-R	20,000	60,000	0.0062
Com-R	20,000	40,000	0.0338
Bis-R	20,000	40,000	0.0289
Cer-R	40,000	80,000	0.0003
Com-R	40000	60,000	<0.0001
Bis-R	40,000	80,000	<0.0001
Cer-R	60000	100000	0.0293
Com-R	60,000	100,000	<0.0001
Bis-R	60,000	80,000	0.0001
Com-R	80,000	100,000	0.0146
Bis-R	80,000	100,000	0.0272
Com-R	100,000	120,000	<0.0001

Abbreviations: Bis-R, bisacryl-resin; Cer-R, ceramic-resin; Com-R, composite-resin.
[a]*All higher number of cycles were different than the lower number of cycles for each row.*
[b]*Bonferroni-corrected* p-*values.*

nature is used in transitioning to a more definite restoration.[28] Bis-R displayed mechanical properties similar to Com-R, which is encouraging considering its indication of use beyond the limited time period of a couple of weeks for a conventional temporary restoration. Moreover, providing an option to use a self-polymerizing, long-term temporary can become a valuable option for temporization during a long-term treatment plan, or simply to be used as a definitive restoration in patients who cannot afford the cost of a more expensive restorative treatment plan. Significant difference in the CS of Cer-R material led to rejecting the null hypothesis.

Wear evaluation using a mastication simulator with a steatite antagonist is a well-documented method.[15] It may provide insight to properties of a relatively new material available for clinical use but lacking proper independent clinical evidence of performance. The wear loss from Bis-R and Com-R exceeded the wear of Cer-R, which was expected due to the composition of the tested materials. As previously mentioned, FS has been reported to show positive correlation with wear.[20] Although Com-R had the greatest flexural strength of the tested materials, it also had the greatest wear loss. The limited wear loss of Cer-R was expected due to the primarily ceramic matrix. The greater the volumetric wear, the smaller the modulus, suggesting that increased elasticity leads to greater wear. However, opposing wear is less severe when the elastic modulus of the material is similar to tooth structure. Therefore, both the wear of the material and the wear of the opposing must be considered thoroughly when choosing an appropriate restorative material. Wear of enamel has been reported to be 0.22 mm^3 in a study testing the wear of enamel with a methodology similar to the one used in this study.[29]

Considering this a standard rate of wear, the Cer-R wear rate is closest to enamel, followed by Bis-R and

Com-R. With these differences in wear resistance amongst the resin-based materials, the null hypothesis was rejected. Noteworthy, Cer-R restorations are usually glazed with a varnish (methyl methacrylate and acrylic resin) layer to enhance color stability and are claimed by the manufacturer to be abrasion-resistant. This layer may delay wear of the Cer-R material and prolong its wear-resistance, as it may take longer to wear through the glaze.

These laboratory tests should be interpreted with care, and making any clinical relevance of such findings can only be confirmed with proper clinical investigation. Unfortunately, clinical trials investigating such properties are scarce. New materials are introduced at a fast rate with very limited to no clinical evidence of their support. Therefore, laboratory testing can provide useful information about a relatively new material with proper testing, given that the results are interpreted with care and consideration of the limitations. Although artificial aging is known to affect the mechanical properties of a resin-based material, time limitations only allowed for fracture toughness and volumetric wear of each material to be determined before and after thermomechanical loading.

CONCLUSIONS

Considering the limitations of this laboratory study, the following conclusions may be drawn:

1. Fracture toughness of Bis-R is lower than Cer-R and Com-R materials. Thermomechanical loading increased the FT of Bis-R to levels comparable to Cer-R while decreasing the FT of Com-R.
2. Flexural strength of Comp-R was higher than other materials, while the FM of Bis-C was significantly lower. Contrarily, the CS of Cer-R was significantly higher than Bis-R and Comp-R, which were comparable.
3. Cer-R was more resistant to wear than the other materials; however, Bis-R and Com-R wore at a rate similar to enamel, with Bis-R slightly more resistant to wear.

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 Performance of Different Universal Adhesive Systems on Several CAD/CAM Restorative Materials After Thermal Aging

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Clinical Relevance

The performance of universal adhesives varied for different CAD/CAM materials. The results of this study may help clinicians elect the best adhesive system for each specific clinical case.

SUMMARY

Objective: To evaluate the microshear bond strength (mSBS) of 10 universal adhesive systems applied on five different CAD/CAM restorative materials, immediately and after thermal aging.

Methods and Materials: Five CAD/CAM materials

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were selected: 1) feldspathic glass ceramic (FeCe); 2) pre-polymerized reinforced resin composite (ReRC); 3) leucite-reinforced glass ceramic (LeGC); 4) lithium disilicate (LiDi); and 5) yttrium-stabilized zirconium dioxide (ZiDi). For each material, 15 blocks were cut into four rectangular sections (6 × 6 × 6 mm; n=60 per group) and processed as recommended by the respective manufacturer. For each indirect material, the following adhesive systems were applied according to the respective manufacturer's instructions: 1) AdheSE Universal [ADU]; 2) All-Bond Universal [ABU]; 3) Ambar Universal [AMB]; 4) Clearfil Universal Bond [CFU]; 5) Futurabond U [FBU]; 6) One Coat 7 Universal [OCU]; 7) Peak Universal Bond [PUB]; 8) Prime&Bond Elect [PBE]; 9) Scotchbond Universal Adhesive [SBU]; 10) Xeno Select [XEN, negative control]. After the application of the adhesive system, cylinder-shaped transparent matrices were filled with a dual-curing resin cement (NX3) and light cured. Specimens were tested in shear mode at 1.0 mm/min (mSBS), after 24 hours and 10,000 thermal cycles (TC). All data were submitted to statistical analysis (α=0.05).

Results: For FeCe, there was no significant decrease in mean mSBS for AMB, FBU, and SBU after TC when compared at 24 hours. For ReRC, AMB and SBU showed higher mean mSBS when compared to CFU and XEN, after 24 hours and TC. For LiDi, FBU and OCU showed higher mean mSBS when compared to CFU and XEN, after 24 hours and TC. For LeGC, AMB and PUB showed higher mean mSBS when compared to XEN, after 24 hours and TC. For ZiDi, OCU and SBU showed higher mean mSBS when compared to XEN, after 24 hours and TC. In addition, PBE and XEN showed the lowest mean mSBS after TC with higher percentage of bond strength reduction.

Conclusions: The mean mSBS among the different universal adhesives varied widely for each CAD/CAM material used. In addition, most universal adhesives underwent a statistically significant bond strength reduction after TC.

INTRODUCTION

Computer-aided design and manufacturing (CAD/CAM) has become an extremely important part of dentistry during the last two decades, as a result of the advances in intra-oral imaging and manufacturing technologies.[1-3] CAD/CAM technology has had a dramatic impact on several disciplines especially in prosthodontics and restorative dentistry, making tooth restoration easier and faster. In addition, it provides immediate chairside restorations without the risk of contamination with temporary cements and the need for provisional restoration.[4,5]

Within the current materials available for CAD/CAM restorations, glass-matrix ceramics and polycrystalline ceramics are the most widely used.[3] Nevertheless, new CAD/CAM ceramics known as resin-matrix hybrid materials have been recently introduced. These include pre-polymerized reinforced resin composite materials and polymer-infiltrated ceramic network (PICN) materials,[6,7] which have some mechanical properties similar to those of glass-matrix ceramics.[7,8]

The cementation procedure of a ceramic restoration is an important step in restorative dentistry for the long-term clinical success. This success depends on two interfaces, dental tissues with resin cement, but also resin cement with the specific restorative material.[9] The intaglio surface of resin-matrix ceramic restorations can be treated with sandblasting or with hydrofluoric acid, followed by a silane coupling agent.[10] On the other hand, sandblasting is not needed for glass-matrix ceramics,[11] as adhesion is achieved through a combination of micromechanical retention upon hydrofluoric acid etching and chemical adhesion provided by the silane coupling agent.[12] In the case of polycrystalline ceramics or glass-free oxide-based ceramics, hydrofluoric acid does not improve bond strengths, since these substrates do not contain a glass matrix.[13] Hydrofluoric acid may be effective on zirconia, but it would require longer application time, higher concentration, and/or a higher temperature, which makes it clinically unfeasible.[14,15]

In this context, several protocols have been advocated to achieve durable adhesion to polycrystalline ceramics. Some of the newest protocols include primers or silanes mixed with functional monomers, such as 10-methacryloyloxydecyl dihydrogenphosphate (MDP), in order to increase the potential for chemical interaction (ie, Monobond Plus [MB+], Ivoclar Vivadent, Shaan, Liechtenstein).[9] In addition, most universal adhesives also contain functional monomers such as MDP. The indications for these adhesive systems has been expanded to glass-matrix ceramics, polycrystalline ceramics, and metal alloys without the need for additional primers. However, a recent study showed that the presence of MDP in universal adhesives did not have a significant influence on the microshear bond strengths of several CAD/CAM materials in the immediate time.[12]

Another recent development in dental adhesion is the introduction of silane-containing universal adhesives (ie, Clearfil Universal Bond, Kuraray Noritake Dental, Tokyo, Japan) and Scotchbond Universal Adhesive (3M Oral Care, St Paul, MN, USA), in order to bond glass-rich (through silane) and glass-poor ceramics such as zirconia (through MDP). Although a universal adhesive with silane in the same solution is clinically useful, the effectiveness and long-term stability of silane contained in the universal adhesive is controversial.[16,17]

Accordingly, the effectiveness of universal adhesives with or without MDP, as well as universal adhesives containing silane on different CAD/CAM restorative materials, has not been extensively studied. In addition, there are few studies that investigated the effect of thermal aging on the interface between universal adhesives and different CAD/CAM indirect materials, and how substrates and universal adhesives behave after aging.

Thus, the aim of the present study was to evaluate the microshear bond strength of several universal adhesive systems on five different CAD/CAM indirect materials, after 24 hours of water storage or after 10,000 thermal cycles. The null hypotheses tested were: 1) a given universal adhesive would not result in different bond strengths between immediate time and after 10,000 thermo-cycles, in all five indirect materials; and 2) universal adhesives would not result in different bond strengths for each indirect substrate.

METHODS AND MATERIALS

Sample Size Calculation

To calculate the sample size, we considered the data of Scotchbond Universal (Scotchbond Universal Adhesive, 3M Oral Care), also known as Single Bond Universal in some countries. Means and standard deviations of bond strength of this adhesive system to zirconia as reported in the literature is 29.0 ± 1.1 MPa.[18] According to the free website, www.sealedenvelope.com, the minimum sample size required was six ceramic blocks in each group in order to detect a difference of 7 MPa among the tested groups, using a two-sided test with an α of 0.05 and a power of 80%.

Specimen Preparation

Five CAD/CAM materials were selected: 1) feldspathic glass ceramic (FeCe, Vitablocs RealLife, VITA Zahnfabrik; Bad Säckingen, Germany); 2) pre-polymerized reinforced resin composite (ReRC, Lava Ultimate CAD/CAM Restorative, 3M Oral Care); 3) leucite-reinforced glass ceramic (LeGc, IPS Empress CAD, Ivoclar Vivadent); 4) lithium disilicate (LiDi, IPS e.max CAD, Ivoclar Vivadent) and; 5) yttrium-stabilized zirconium dioxide (ZiDi, Ceramill Zi, Amann Girrbach, Koblach, Austria).

A total of 75 CAD/CAM blocks, 15 for each material, were used. For each material, the blocks (12 × 12 × 6 mm) were cut into four rectangular sections (6 × 6 × 6 mm; n=60 per group), using a diamond disk in slow speed (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling.[12] When applicable, the specimens were fired following the crystallization program recommended by the respective manufacturer. ZiDi specimens were sintered according to the manufacturer's recommended protocol (Table 1).

Experimental Design

The specimens (n=60 for each indirect material) were randomly assigned (http://www.sealedenvelope.com) into 10 groups according to the adhesive system used: 1) Adhese Universal (ADU, Ivoclar Vivadent, also known as Tetric N-Bond Universal, Ivoclar Vivadent, Schaan, Liechtenstein); 2) All-Bond Universal (ABU, Bisco, Schaumburg, IL, USA); 3) Ambar Universal (AMB, FGM Prod Odont, Joinville, SC, Brazil); 4) Clearfil Universal Bond (CFU, Kuraray Noritake Dental); 5) Futurabond U (FBU, VOCO, Cuxhaven, Germany); 6) One Coat 7 Universal (OCU, Coltene, Altstätten, Switzerland); 7) Peak Universal Bond (PUB, Ultradent Products, South Jordan, UT, USA); 8) Prime&Bond Elect (PBE, Dentsply Sirona, Milford, DE, USA); 9) Scotchbond Universal Adhesive (SBU, 3M Oral Care, also known as Single Bond Universal in some countries); and 10) Xeno Select (XEN, Dentsply Sirona, also known as Prime&Bond One Select in some countries, Konstanz, Germany). XEN was used as a negative control, as the respective manufacturer does not recommend XEN for indirect restorations due to its low pH. The composition, application mode, and batch numbers are described in Table 2.

Microshear Bond Strength (μSBS)

The specimens were placed inside polyvinyl chloride (PVC) previously filled with acrylic resin (AutoClear, DentBras, Pirassununga, SP, Brazil), leaving a distance of 3 mm between the free surface of the ceramic and the top of the PVC ring. The description of the materials and their respective surface treatments are displayed in Table 1. Monobond Plus (MB+, silane + MDP solution, Ivoclar Vivadent) was used in all substrates prior to the application of universal adhesives, in order to standardize the experimental procedure. Then, the universal adhesives were applied according to the respective manufacturer's instructions (Table 2). A single operator performed all bonding procedures.

Table 1: *Materials Used, Composition, and Surface Treatment*

Material	Composition	Surface Treatment
Feldspathic glass ceramic (FeCe, Vitablocs RealLife, Vita)	SiO_2, Al_2O_3, K_2O, Na_2O, CaO, TiO_2	5% Hydrofluoric acid etching applied for 60 s (Vita Ceramics Etch; batch 42530). Rinsed with air-jet drying for 30 s. Ultrasonically clear with distilled water for 180 s. Silane Solution: Monobond Plus (Ivoclar Vivadent) applied with a brush and allowed to react for 60 seconds. Subsequently, the excess was dispersed with a strong stream of air to ensure the solvent evaporation.
Indirect resin composite (ReRC, Lava Ultimate CAD/CAM, 3M Oral Care)	Bis-GMA, UDMA, Bis-EMA, TEGDMA, 80wt% SiO_2 (20 nm) and ZrO_2 (4-11 nm) particles, aggregated ZrO_2/SiO_2 clusters	Sandblast with Al_2O_3, <50 μm (2 bar, until entire bonding surface appears matte. Ultrasonically clear with distilled water for 180 s. Remove sand with alcohol. Dry with oil-free air Silane Solution: Monobond Plus (Ivoclar Vivadent) applied with a brush and allowed to react for 60 s. Subsequently, the excess was dispersed with a strong stream of air to ensure the solvent evaporation.
Leucite-reinforced glass-ceramic (LeGC, IPS Empress CAD, Ivoclar Vivadent)	SiO_2, Al_2O_3, K_2O, Na_2O, other oxides, pigments	5% hydrofluoric acid etching for 60 s. Rinsed with water for 30 s. Ultrasonically cleaned with distilled water for 180 s. Silane Solution: Monobond Plus (Ivoclar Vivadent) applied with a brush and allowed to react for 60 s. Subsequently, the excess was dispersed with a strong stream of air to ensure solvent evaporation.
Lithium disilicate glass-ceramic (LiDi, IPS e.max CAD, Ivoclar Vivadent)	SiO_2, Li_2O, K_2O, P_2O_5, ZrO_2, ZnO, other oxides, coloring oxides	Crystallization in furnace (Programat P300, Ivoclar Vivadent) at 840 - 850ºC for 20 - 31 min. 5% hydrofluoric acid etching for 20 s.[a] Rinsed with water for 30 s. Dried with oil-free air for 30 s. Ultrasonically cleaned with distilled water for 180 s. Silane Solution: Monobond Plus (Ivoclar Vivadent) applied with a brush and allowed to react for 60 s. Subsequently, the excess was dispersed with a strong stream of air to ensure solvent evaporation.
Yttrium-stabilized zirconium dioxide (ZiDi, Ceramill Zi, Amann Girrbach AG)	$ZrO_2 + HfO_2 + Y_2O_3$: >99% Y_2O_3: 4.5 - 5.6 % HfO_2: < 0.5% Al_2O_3: <0.5%	Sintered in a furnace (Ceramill Therm, Amann Girrbach, Curitiba, PR, Brazil) using a universal program (8ºC/min from 200ºC to 1450ºC), 2 h at a fixed temperature of 1450ºC, and the correct cooling time. Sandblasted <50-μm Al_2O_3 particles (2.8 bar, 7s). Ultrasonically cleaned with distilled water for 180 s. The surface was thoroughly rinsed (5 ml) with ethyl alcohol (70%). Dry with oil-free air for 30 s. Silane Solution: Monobond Plus (Ivoclar Vivadent) applied with a brush and allowed to react for 60 s. Subsequently, the excess was dispersed with a strong stream of air to ensure solvent evaporation.

Abbreviations: Bis-GMA, bisphenol A diglycidylether methacrylate; UDMA, urethane dimethacrylate; Bis-EMA, ethoxylated bisphenol-A dimethacrylate; TEGDMA, triethylene glycol dimethacrylate.

[a]*Condac Porcelain Etch 5% (FGM Prod Odont Ltda, Joinville, SC, Brazil)*

Ten polyethylene transparent Tygon tubes (Tygon Medical Tubing Formulations 54-HL, Saint Gobain Performance Plastics, Akron, OH, USA) with an internal diameter of 0.8 mm and a height of 0.5 mm, which were positioned over the substrate. After that, in order to standardize the experimental procedure, an amine-free dual-curing resin cement (NX3, Kerr, Orange, CA, USA) was used to avoid the possible incompatibility of universal adhesives when in contact with dual-cured resin cements. This dual-curing resin cement was carefully packed inside each tube, and a clear Mylar matrix strip was placed over the filled Tygon tube and pressed gently into place. The resin cement was light cured for 20 seconds using an LED light-curing unit set at 1,200 mW/cm^2 (Radii-cal, SDI Limited, Bayswater, Victoria, Australia). A radiometer (Demetron L.E.D. Radiometer, Kerr Sybron Dental Specialties, Middleton, WI, USA) was used to check the light intensity after every five specimens. These procedures were carried out under magnifying loupes.

After storage of the specimens in distilled water for 24 hours at 37°C the Tygon tubes were carefully removed with a blade, exposing the cement cylinders; each specimen was examined under a stereomicroscope at 10× magnification, the bonded cylinder was discarded if there was evidence of porosities or gaps at the interface. Half of the specimens were tested immediately, and the other half tested after 10,000 thermal cycles in distilled water between water baths held at 5 and 55 °C, with a dwell time of 1 minute.[19]

The specimens were attached to a shear-testing jig (Odeme Biotechnology; Joaçaba, SC, Brazil) and tested in a universal testing machine (Kratos IKCL 3-USB, Kratos Equipamentos Industriais; Cotia, São Paulo, Brazil). Each specimen was mounted in the universal testing machine and a thin orthodontic wire (0.2 mm diameter) was looped around the base of each composite cylinder. The orthodontic wire contacted the composite cement cylinder along half of its circumference. The setup was kept aligned (resin/substrate interface, the wire loop, and the center of the load cell) to ensure the correct orientation of the shear forces.[20] The crosshead speed was set at 1 mm/min until failure.

The μSBS values (MPa) were calculated by dividing the load at failure by the surface area (mm^2). After testing, the specimens were examined under an optical microscope (SZH-131, Olympus; Tokyo, Japan) at 100× magnification to define the location of the bond failure. The failure mode was classified as cohesive failure exclusively in resin cement (CR), cohesive failure exclusively in the ceramic or CAD/CAM indirect resin composite (CC), adhesive/mixed (A/M) failure at the cement/ceramic interface, which included cohesive failure of the ceramic and/or indirect resin composite, resin cement, and adhesive material. Also, the premature failures were recorded.

Statistical Analysis

The data were first analyzed using the Kolmogorov-Smirnov test to assess whether the data followed a normal distribution, and Bartlett's test for equality of variances to determine if the assumption of equal variances was valid. After confirming the normality of the data distribution and the equality of the variances, the μSBS (MPa) data were subjected to appropriate statistical analysis. The μSBS of all specimens from the same individual indirect specimens were averaged for statistical purposes. Two-way ANOVA was used to analyze the μSBS data for each indirect material (adhesive vs storage time). After that, the Tukey's post-hoc test was used at $\alpha = 0.05$.

RESULTS

For each indirect material, 30 cylinders were tested at each evaluation time. In the immediate time, the majority of specimens for all indirect materials showed adhesive/mixed failures (Table 3). For some materials there were some cohesive fractures of cement or in the indirect material (Table 3). However, after thermocycling, specimens showed adhesive/mixed failures for all indirect materials, with only a few cohesive fractures of cement or indirect material (Table 3). For ZiDi it is worth mentioning that 16.7% premature failures occurred with CFU, 40% with PBE, and 60% with XEN after thermocycling (Table 3).

For FeCe, the interaction between main factors were statistically significant ($p<0.0001$; Table 4). The application of ADU, ABU, AMB, and PUB resulted in statistically significant higher mean μSBS when compared with those of CFU, OCU, and XEN in the immediate time ($p<0.0001$; Table 4). However, after thermocycling, only AMB, FBU, and SBU showed higher mean μSBS when compared to the remainder of the universal adhesives tested (ADU, ABU, CFU, OCU, PUB, PBE, and XEN; $p<0.0001$; Table 4). XEN showed the lowest mean μSBS after thermocycling; its reduction of more than 40% of bond strength similar only to PBE ($p<0.0001$; Table 4). There was no significant decrease in mean μSBS for AMB, FBU, and SBU after thermocycling when compared to the immediate time ($p>0.05$; Table 4).

For ReRC, the interaction between main factors was statistically significant ($p<0.0004$; Table 5). In the immediate time, statistically higher mean μSBS were observed when ABU, AMB, FBU and SBU were compared with those of ADU, CFU, OCU, PUB and

Table 2: *Adhesive System (Batch Number), Composition, and Application Mode of the Adhesive According the Manufacturer's Instructions*

Adhesive (Batch Number)	Composition	Application Mode[a]
Adhese Universal, ADU, Ivoclar Vivadent (UO2709) pH = 2.5 to 3.0	HEMA, 10-MDP, bis-GMA, MCAP, D3MA, ethanol, water, highly dispersed silicon dioxide and CQ	1. Apply one coat of adhesive for 20 s. 2. Gently air thin for 5 s. 3. Light cure for 10 s at 1,200 mW/cm^2.
All-Bond Universal, ABU, Bisco (1500002859) pH = 3.1 to 3.2	HEMA, 10-MDP, bis-GMA, ethanol, water, initiators	1. Apply one coat of adhesive. 2. Evaporate excess solvent by thoroughly air drying with an air syringe for at least 10 s until no visible movement of the material is observed. The surface should have a uniform, glossy appearance. 3. Light cure for 10 s at 1,200 mW/cm^2.
Ambar Universal, AMB, FGM (210415) pH = 2.6 to 3.0	Methacrylate monomers (UDMA and 10-MDP), photo-initiators, co-initiators, stabilizers, inert silica nanoparticles, ethanol, water	1. Apply two coats vigorously by rubbing the adhesive for 20 s (10 s each). 2. Gently air dry for 10 s to evaporate the solvent. 3. Light cure for 10 s.
Clearfil Universal Bond, CFU, Kuraray Noritake Dental (CR0002) pH = 2.3	Bis-GMA, HEMA, ethanol, 10-MDP, hydrophilic aliphatic dimethacrylate, colloidal silica, CQ, silane coupling agent, accelerators, initiators, water	1. Apply bond and leave it in place for 5 s. 2. Dry by blowing with a mild air stream for 5 s until the mixture does not move. 3. Light cure for 10 s at 1,200 mW/cm^2.
Futurabond U, FBU, Voco (1346518) pH = 2.3	HEMA, bis-GMA, HEDMA, 10-MDP, UDMA, catalyst, silica nanoparticles, ethanol, water	1. Apply the adhesive with microbrush for 20 s. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely. 3. Light cure for 10 s at 1,200 mW/cm^2.
One Coat 7 Universal, OCU, Coltene (F96836) pH = 2.8	HEMA, hydroxypropylmethacrylate, MMA-modified polyacrylyic acid, UDMA, amorphous silica, MDP, ethanol, water	1. Rub with a disposable brush for 20 s. 2. Dry gently with oil-free compressed air for 5 s. 3. Light cure for 10 s at 1,200 mW/cm^2.
Peak Universal Bond, PUB, Ultradent Products (BB7D7) pH = 2.0	Bis-GMA, ethyl alcohol, 0.2% chlorhexidine di(acetate), methacrylic acid, HEMA, 7.5% filler.	1. Apply a puddle coat of Peak Universal Bond with gentle agitation for 10 s. 2. Gently dry with clean air for at least 5 s; surface should have a uniform, glossy appearance. 3. Light cure for 10 s at 1,200 mW/cm^2.
Prime&Bond Elect, PBE, Dentsply Sirona (130811) pH = 2.5	Mono-, di- and trimethacrylate resins, PENTA diketone, organic phosphine oxide, stabilizers, cetylamine hydrofluoride, acetone, water.	1. Apply generous amount of adhesive thoroughly to all surface and leave undisturbed for 20 s. 2. Gently dry with clean air for at least 5 s. Surface should have uniform, glossy appearance. 3. Light cure for 10 s at 1,200 mW/cm^2.

Table 2: *Adhesive System (Batch Number), Composition, and Application Mode of the Adhesive According the Manufacturer's Instructions (cont.)*

Scotchbond Universal, SBU, 3M Oral Care (523652) pH = 2.7	10-MDP, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, nanofiller, ethanol, water, initiators, silane.	1. Apply the adhesive and leave undisturbed for 20 s. 2. Direct a gentle stream of air over the liquid for about 5 s until there is no longer any movement and the solvent is evaporated completely. 3. Light cure for 10 s at 1,200 mW/cm^2.
Xeno Select, XEN, Denstply Sirona (1401001210) pH = 1.6	Bifunctional acrylates, acidic acrylate, functionalized phosphoric acid ester (ethyl 2-[5-dihydrogen phosphoryl-5,2-dioxapentyl]acrylate), water, tert-butyl alcohol, initiator (camphorquinone), co-initiator (DMABN), stabilizer.	1. Apply a generous amount of adhesive to thoroughly wet all surfaces and agitate for 20 s. 2. Gently dry with clean air for a least 5 s. Surface should have a uniform, glossy appearance. 3. Light cure for 10 s at 1,200 mW/cm^2.
NX3 Nexus, Kerr (6108657)	Bis-GMA, UDMA, EBPADMA, TEGDMA, HEMA, activators, stabilizers, ytterbium fluoride, fumed silica, barium aluminoborosilicate.	1. Carefully pack resin cement inside each tube and place a clear Mylar matrix strip over the filled Tygon tube and gently pressed into place. 2. Light cure for 20 s at 1,200 mW/cm^2.
Monobond Plus, MB+ Ivoclar Vivadent (S31153) pH = 3.1	Ethanol, 3-trimethoxysilypropyl methacrylate, methacrylated phosphoric acid ester (10-MDP) and disulfide acrylate.	1. Apply with a brush and allow to react for 60 s. 2. Blow with a strong stream of air to ensure solvent evaporation.

Abbreviations: 10-MDP, methacryloyloxydecyl dihydrogen phosphate; bis-GMA, bisphenol glycidyl methacrylate; MCAP, methacrylated carboxylic acid polymer; CQ, camphorquinone; D3MA, decanediol dimethacrylate; DMABN, 4- (dimethylamino)benzonitrile; HEDMA, hexamethylene dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; PENTA, dipentaerythritol penta acrylate monophosphate; UDMA, urethanedimethacrylate; EBPADM, (ethoxylated bisphenol A-dimethacrylate); TEGDMA, (trieth-ylene glycol dimethacrylate).

[a] *The intensity of light curing was standardized for all materials.*

XEN (p<0.0004; Table 5). After thermocycling, AMB, OCU, and SBU resulted in statistically higher mean μSBS when compared to those of CFU and XEN, which showed 49.4% and 56.1% of bond strength reduction, respectively, after thermocycling (p<0.0003; Table 5).

When the universal adhesives were evaluated on the LiDi substrate, the interaction between main factors was statistically significant (p<0.0003; Table 5). In the immediate time, statistically higher mean μSBS were found for AMB, FBU, OCU, and PUB compared to those of CFU, PBE, and XEN (p<0.0003; Table 5). After thermocycling, FBU, OCU, and SBU showed higher mean μSBS when compared to CFU and XEN (p<0.0003; Table 5). CFU and XEN showed the highest percentage of μSBS reduction with 45.5% and 48.3%, respectively (Table 5).

For LeGC, the interaction between main factors was statistically significant (p<0.0004; Table 6). Statistically higher mean μSBS values in the immediate time were observed only for AMB, PUB, and SBU compared to those of OCU and XEN (p<0.0004; Table 6). After thermocycling, AMB, FBU, and PUB showed higher mean μSBS when compared to XEN, which showed the lowest mean μSBS after thermocycling (p<0.0004; Table 6).

The interaction between main factors was statistically significant (p<0.0001; Table 6) when the universal adhesives were evaluated in the yttrium-stabilized zirconium dioxide (ZiDi). In the immediate time, the highest mean μSBS values were measured for ADU, ABU, OCU, and SBU, which were statistically higher compared to those of XEN (p<0.00001; Table 6). After thermocycling, AMB, OCU, and SBU showed higher mean μSBS when compared to those of ADU, ABU, CFU, FBU, PUB, PBE, and XEN. However, two universal adhesives (PBE and XEN) showed the

Table 3: *Number (%) of Specimens According to Fracture Mode*

Immediate															
Adhesive System	**Feldspathic Glass Ceramic (FeCe) -Vita Mark II**			**Indirect Resin Composite (InRC) -Lava Ultimate CAD/CAM**			**Leucite-reinforced Glass-ceramic (LeGC) - IPS Empress CAD**			**Lithium Disilicate Glass-ceramic (LiDi) -IPS e.max CAD**			**Yttrium-stabilized Zirconium Dioxide (ZiDi)- Ceramill Zi**		
	A/M	**CR**	**CC**	**A/M**	**CR**	**CC**	**A/M**	**CR**	**CC**	**A/M**	**CR**	**CC**	**A/M**	**CR**	**CC**
ADU	30 (100)	0 (0)	0 (0)	29 (97)	0 (0)	1 (3)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
ABU	16 (53)	2 (7)	12 (40)	21 (70)	0 (0)	9 (30)	20 (67)	0 (0)	10 (33)	28 (93)	2 (7)	0 (0)	28 (93)	2 (7)	0 (0)
AMB	18 (60)	2 (7)	10 (33)	20 (67)	1 (3)	9 (30)	22 (74)	0 (0)	8 (26)	30 (100)	0 (0)	0 (0)	28 (93)	2 (7)	0 (0)
CFU	27 (90)	0 (0)	3 (10)	25 (83)	0 (0)	5 (17)	17 (57)	1 (3)	12 (40)	27 (90)	3 (10)	0 (0)	28 (93)	2 (7)	0 (0)
FBU	18 (60)	3 (10)	9 (30)	20 (67)	0 (0)	10 (33)	20 (67)	0 (0)	10 (33)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
OCU	27 (90)	0 (0)	3 (10)	26 (87)	1 (3)	3 (10)	21 (70)	2 (7)	7 (23)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
PUB	21 (70)	3 (10)	6 (20)	22 (73)	0 (0)	8 (27)	20 (67)	2 (7)	8 (26)	28 (93)	2 (7)	0 (0)	30 (100)	0 (0)	0 (0)
PBE	20 (67)	0 (0)	10 (33)	26 (87)	0 (0)	4 (13)	21 (70)	0 (0)	9 (30)	26 (87)	4 (13)	0 (0)	29 (97)	1 (3)	0 (0)
SBU	24 (80)	0 (0)	6 (20)	19 (63)	0 (0)	11 (37)	22 (74)	0 (0)	8 (26)	26 (87)	4 (13)	0 (0)	28 (93)	2 (7)	0 (0)
XEN	21 (70)	0 (0)	9 (30)	26 (87)	0 (0)	4 (13)	22 (74)	1 (3)	7 (23)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
After Thermocycling															
ADU	29 (97)	0 (0)	1 (3)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
ABU	26 (87)	0 (0)	4 (13)	28 (93)	0 (0)	2 (7)	26 (87)	0 (0)	4 (13)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
AMB	25 (83)	0 (0)	5 (17)	29 (97)	0 (0)	1 (3)	27 (90)	0 (0)	3 (10)	29 (97)	0 (0)	1 (3)	30 (100)	0 (0)	0 (0)
CFU	28 (93)	0 (0)	2 (7)	30 (100)	0 (0)	0 (0)	25 (83)	0 (0)	5 (17)	30 (100)	0 (0)	0 (0)	25 (83)	0 (0)	0 (0)
FBU	24 (80)	1 (3)	5 (17)	27 (90)	0 (0)	3 (10)	26 (87)	0 (0)	4 (13)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
OCU	29 (97)	0 (0)	1 (3)	30 (100)	0 (0)	0 (0)	27 (90)	0 (0)	3 (10)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
PUB	24 (80)	0 (0)	6 (20)	28 (93)	0 (0)	2 (7)	29 (97)	0 (0)	1 (3)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)
PBE	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	18 (60)	0 (0)	0 (0)
SBU	28 (93)	0 (0)	2 (7)	28 (93)	0 (0)	2 (7)	26 (87)	0 (0)	4 (13)	29 (97)	1 (3)	0 (0)	30 (100)	0 (0)	0 (0)
XEN	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	30 (100)	0 (0)	0 (0)	12 (40)	0 (0)	0 (0)

Abbreviations: ADU, Adhese Universal; ABU, All-Bond Universal; A/M, adhesive/mixed fracture mode; AMB, Ambar Universal; CC, cohesive in indirect restorative material; CFU, Clearfil Universal Bond; CR, cohesive in resin cement; FBU, Futurabond U; OCU, One Coat 7 Universal; PUB, Peak Universal Bond; PBE, Prime & Bond Elect; SBU, Scotchbond Universal; XEN, Xeno Select.

lowest mean μSBS after thermocycling with higher percentage of bond strength reduction (84.6% and 65.5%, respectively; $p<0.00001$; Table 6).

DISCUSSION

The main objective of the present study was to evaluate whether the mean μSBS of different universal adhesives would decrease after thermocycling when applied on materials used for indirect restorations. Although several universal adhesives are indicated for luting procedures, there are no published studies to our knowledge that have evaluated several universal adhesives applied on a wide number of materials used for indirect restorations.

A recent meta-analysis of universal adhesives used for indirect procedures showed that the majority of the studies evaluated up to four universal adhesives (ABU, ADU, CFU, and SBU) applied to one or two indirect substrates.[21] However, the majority of studies evaluated universal adhesives against lithium disilicate glass ceramics[17,22,23] or yttrium-stabilized zirconium dioxide.[17,18,24] Only a few studies have evaluated the bond strength to glass ceramics, leucite-reinforced[25] or indirect composite.[26] Therefore, it is relevant to test the longevity of several different universal adhesives *in vitro* applied on the recent CAD/CAM materials for indirect restorations.

Table 4: *Mean ± Standard Deviation of Microshear Bond Strength (μSBS) of Universal Adhesives Bonded to Feldspathic Glass Ceramic*[a]

Adhesive System	Feldspathic Glass Ceramic (FeCe)		
	Immediate	After Thermocycling	Bond Strength Reduction (%)
ADU	32.2 ± 1.5 A	21.1 ± 3.3 D	34.4
ABU	29.3 ± 1.6 A	21.9 ± 2.6 D	25.3
AMB	28.5 ± 1.3 AB	25.8 ± 4.1 B	9.5
CFU	23.2 ± 1.9 C	16.7 ± 2.8 D	28.0
FBU	27.1 ± 1.8 B	24.2 ± 2.6 BC	10.7
OCU	24.2 ± 1.3 C	20.8 ± 3.2 D	14.1
PUB	28.2 ± 1.1 AB	21.4 ± 2.9 D	24.1
PBE	25.2 ± 1.6 BC	17.5 ± 1.0 DE	30.6
SBU	26.3 ± 1.3 BC	22.3 ± 3.1 C	15.2
XEN	22.5 ± 1.6 C	13.4 ± 0.8 E	40.4

Abbreviations: ADU, Adhese Universal; ABU, All-Bond Universal; AMB, Ambar Universal; CFU, Clearfil Universal Bond; FBU, Futurabond U; OCU, One Coat 7 Universal; PUB, Peak Universal Bond; PBE, Prime & Bond Elect; SBU, Scotchbond Universal; XEN, Xeno Select.
[a]*Different letters indicate statistically significant differences (two-way ANOVA, Tukey's test,* $p<0.05$*).*

Table 5: *Mean ± Standard Deviation of Microshear Bond Strength (μSBS) of Universal Adhesives Bonded to Pre-polymerized Reinforced Resin Composite and Lithium Disilicate Glass-ceramic*[a]

Adhesive System	Pre-polymerized Reinforced Resin Composite (ReRC)			Lithium Disilicate Glass-ceramic (LiDi)		
	Immediate	After Thermocycling	Bond Strength Reduction (%)	Immediate	After Thermocycling	Bond Strength Reduction (%)
ADU	25.3 ± 1.5 B	16.8 ± 1.8 DE	33.6	25.5 ± 1.4 b	16.8 ± 2.9 d	34.1
ABU	30.4 ± 1.7 A	17.2 ± 2.8 DE	43.3	25.9 ± 1.5 b	16.2 ± 1.5 d	37.5
AMB	28.1 ± 1.9 A	18.2 ± 2.8 D	35.2	28.2 ± 1.2 ab	16.4 ± 1.8 d	41.8
CFU	24.3 ± 1.1 B	12.3 ± 1.3 E	49.4	23.1 ± 1.8 c	12.6 ± 2.2 e	45.5
FBU	27.0 ± 1.7 A	16.6 ± 2.0 DE	38.5	27.2 ± 1.2 ab	19.6 ± 2.7 c	28.0
OCU	24.2 ± 1.1 B	18.9 ± 2.2 D	21.9	29.0 ± 1.4 a	18.2 ± 2.6 cd	37.3
PUB	24.1 ± 1.2 B	17.4 ± 2.7 DE	27.8	29.0 ± 1.2 a	17.7 ± 1.7 d	39.0
PBE	26.2 ± 1.6 AB	15.2 ± 2.3 DE	42.0	22.2 ± 1.7 c	15.2 ± 2.0 de	40.2
SBU	30.1 ± 1.9 A	19.0 ± 2.3 D	36.8	25.4 ± 1.4 b	19.7 ± 2.6 c	32.5
XEN	24.6 ± 1.2 B	10.8 ± 1.4 F	56.1	17.2 ± 1.3 cd	8.9 ± 2.5 f	48.3

[a]*Different uppercase (ReRC) and lowercase (LiDi) letters indicate statistically significant differences for each restorative material (two-way ANOVA, Tukey's test,* $p<0.05$*).*

Despite some exceptions (AMB, FBU, and SBU bonding to FeCe), all of the universal adhesives showed a reduction in mean μSBS for all indirect restorative materials when submitted to thermocycling. The reduction in mean μSBS after thermal fatigue may be associated with the small molecular size and high molar concentration of water, which allows penetration of the nano-size spaces between polymer chains or clusters around functional groups that are capable of hydrogen bonding.[27] This phenomenon could result in a decrease in thermal stability and polymer plasticization.[28] In addition, the temperature changes to which the specimens are subjected increase the coefficient of thermal expansion at the adhesive/ceramic interface leading to a premature failure and/or lower μSBS values, due to the loss of chemical retention.[28] This

Table 6: *Mean ± Standard Deviation of Microshear Bond Strength (μSBS) of Universal Adhesives Bonded to Leucite-reinforced Glass-ceramic and Ytrium-stabilized Zirconium Dioxide*[a]

Adhesive System	Leucite-reinforced Glass-ceramic (LeGC)			Yttrium-stabilized Zirconium Dioxide (ZiDi)		
	Immediate	After Thermocycling	Bond Strength Reduction (%)	Immediate	After Thermocycling	Bond Strength Reduction (%)
ADU	27.2 ± 1.7 B	14.5 ± 2.7 D	46.7	32.1 ± 1.5 a	15.4 ± 1.5 de	52.1
ABU	28.7 ± 1.7 AB	14.2 ± 2.2 D	50.5	33.4 ± 1.2 a	17.9 ± 1.9 d	46.5
AMB	28.9 ± 2.0 A	18.4 ± 3.1 C	39.1	30.6 ± 2.0 ab	20.0 ± 2.1 c	34.2
CFU	26.2 ± 1.9 B	12.0 ± 1.8 DE	54.2	30.7 ± 1.7 ab	13.2 ± 1.5 e	57.0
FBU	27.6 ± 1.5 B	16.2 ± 3.2 CD	41.3	29.2 ± 1.3 b	17.7 ± 1.1 d	39.4
OCU	20.3 ± 1.3 C	13.2 ± 2.8 DE	35.0	33.1 ± 1.4 a	20.0 ± 2.8 c	39.6
PUB	25.2 ± 1.6 A	15.0 ± 3.4 CD	40.5	30.2 ± 1.6 ab	15.8 ± 1.9 de	47.7
PBE	26.4 ± 1.4 B	12.7 ± 2.6 DE	51.9	31.5 ± 1.5 ab	4.7 ± 0.7 f	84.6
SBU	30.2 ± 1.9 A	14.7 ± 2.2 D	51.3	32.6 ± 1.1 a	20.7 ± 3.2 c	36.5
XEN	18.1 ± 2.0 C	10.0 ± 2.2 E	44.8	22.3 ± 0.9 c	7.7 ± 4.1 f	65.5

[a]*Different uppercase (ReRC) and lowercase (LiDi) letters indicate statistically significant differences for each restorative material (two-way ANOVA, Tukey's test,* $p<0.05$*).*

could explain the number of adhesive/mixed failures for all indirect materials after thermocycling, with only a few cohesive fractures of cement or indirect material. For ZiDi it is worth mentioning that 16.7% of premature failures occurred with CFU, 40% with PBE, and 60% with XEN after thermocycling (Table 3).

However, the decrease in mean μSBS was not similar for all universal adhesives when evaluated in different indirect materials. Universal adhesives were developed based on the idea of several manufacturers to include acidic functional monomers, such as MDP, in the composition of these adhesives. The addition of acidic functional monomers provides them with a versatility to adhere to dental substrates of different characteristics, such as enamel and dentin, in addition to other substrates including glass-matrix ceramics, oxide-based ceramics, and metal alloys without the need for additional primers.[25,29]

However, some recent studies showed that the presence of MDP in universal adhesives did not have a significant influence on bond strengths when evaluated in the immediate time.[12,30] In fact, several other acidic functional monomers, such as PENTA, MCAP and D3MA (Table 1), could be added to improve the bonding to direct and indirect materials.[31]

In a previous study, PUB, a MDP-free universal adhesive, was the only adhesive for which the mean μSBS reached the highest ranking of statistical significance among all adhesives for all five CAD/CAM indirect materials after 24 hours of storage in distilled water.[12] At that time, the authors attributed these results to the higher viscosity of PUB compared to that of other universal adhesives, which might be responsible for better physical properties. However, in the current study, PUB showed an intermediary behavior in terms of μSBS after thermocycling, with a reduction of 35.8% when all indirect materials are evaluated together. This can be explained because it is not possible to find the presence of any functional monomer within the composition of PUB. Actually, methacrylic acid is a common component of several adhesives without any specific functionality in terms of bonding.[32] According to the respective manufacturer, PUB contains diacetate of chlorhexidine in its composition, mainly because the addition of this compound helps prevent dentin bonding degradation in the adhesive interface *in vitro*.[33] However, there is no evidence that the use of chlorhexidine could improve the bonding to indirect materials. Future studies need to be done to evaluate this hypothesis.

Two other MDP-free universal adhesives were evaluated in the present study: PBE and XEN. Both showed one of the highest reductions in mean μSBS (49.8% and 47.4%, respectively). Both materials contain PENTA or a phosphoric acid ester group in the functionalized monomer very similar to PENTA.[34] Chen and others[35] observed that primers containing 15 and 20 wt% PENTA increased the binding affinity with ZiDi, via the formation of Zr-O-P bond, when compared to a primer containing MDP. Unfortunately, the exact concentration of PENTA or derivates in PBE and XEN are a property of the respective

manufacturer. However, when commercial universal adhesives containing PENTA were compared to commercial universal adhesives containing MDP, lower bond strength to Lidi and ZiDi were observed for the former.[18,22,24] According to Elsayed and others,[24] this could be related to the increased viscosity of the PENTA-containing primer as a result of the presence of five vinyl groups, which may hinder the ability of the primer to establish a strong chemical bond, mainly to ZiDi.[35,36] This mechanism may explain the worst behavior of PBE and XEN in terms of bond strength reduction (84.6% and 65.5%, respectively) when evaluated on ZiDi. In general, intermediary results were obtained for MDP-free universal adhesives when compared to MDP-containing adhesives.

It is worth mentioning that PBE is the only acetone-based universal adhesive. Ethanol, water, or a mix of them are commonly used as solvents of universal adhesive. Acetone has a higher vapor pressure than ethanol and water, which may reduce the time required for evaporation compared to ethanol.[32] A recent study showed that a longer evaporation time than that recommended by the respective manufacturer was required for PBE.[37] This may occur because of the high acetone content in PBE, which may hinder an adequate solvent evaporation after application. This can leave residual solvent in the adhesive resin, which results in porosities in the cured adhesive layer,[38] which has been corroborated by several authors when acetone-based universal adhesives were applied under indirect substrates.[18,22,24]

Also, the pH of universal adhesives seems to play an important role in the bond strengths to different substrates. XEN has the lowest pH among universal adhesives used in this study (pH=1.6)[39] while PBE has a pH around 2.5.[37] These factors might be responsible for the lower adhesion capability of PBE and XEN. However, it is worth mentioning that the respective manufacturer does not recommend XEN for indirect restorations.

We should also point out that an MDP-containing silane was applied on the indirect materials surface prior to the universal adhesives tested. Several studies have shown that the use of an MDP - containing silane improves the chemical interaction when associated with MDP-containing universal adhesives applied under glass ceramics, leucite-reinforced,[25] lithium disilicate[22,23] and yttrium-stabilized zirconium dioxide surfaces.[18,40] For glass ceramics, methacrylate groups within the adhesive can copolymerize with silane molecules[41] and silanol groups produced by the corresponding methoxy groups can react with the glass ceramic surface.[42,43] In the case of yttrium-stabilized zirconium dioxide, MDP may also react with zirconia through hydroxyl groups present both on the MDP molecule and the zirconia surface.[44-46] On the other hand, the application of a second adhesive coating may protect the surface of the MDP-containing silane, maintaining the bonding of silane to lithium disilicate[22] and yttrium-stabilized zirconium dioxide.[18]

Despite the presence of MDP in the majority of universal adhesives evaluated, several differences were observed between MDP-containing adhesives evaluated. These differences could be explained based on the composition of the different adhesives. For example, considering the concept of versatility to different substrates, at least two silane-containing universal adhesives (ie, CFU and SBU) were launched in the market. According to the respective manufacturers, the silane helps improve the bonding to glass-matrix ceramics, such as feldspathic, leucite-reinforced, and lithium disilicate.

However, some concerns have been raised in the literature regarding this issue. It is generally understood that acidic pre-hydrolyzed silane coupling agents have a relatively short shelf life.[47] This occurs due to the hydrolysis and self-condensation of silane being affected by the pH value of a solution. Generally, the silane used in dentistry has a pH between 4 and 5.[48] On the other side, the pH of CFU is 2.3 and the pH of SBU is 2.7.[12] Therefore, it is unlikely that the incorporation of silane increases the bond strengths for these universal adhesives.[17] In fact, this reinforces the idea that a previous application of a silane-based primer is crucial for current silane-containing universal adhesives.[18,22,43] According to Cuevas-Sanchez and others,[21] there are currently three universal adhesives for which the respective manufacturers indicate that the use of a separate primer for adhesion to silicate ceramics is not necessary (CFU, FBU, and SBU).

However, a closer view of the results in our study showed a lower performance of CFU when compared with SBU in four of the five substrates after thermocycling, which is supported by several studies.[42,49] This may be explained by the presence of silane and a high concentration of Bis-GMA in the composition of CFU (15%-35%) in comparison with the concentration of Bis-GMA in SBU (15%-25%). The two components coexisting in one bottle may have a negative influence on the efficiency of a universal adhesive. Chen and others[50] reported that the incorporation of Bis-GMA monomer significantly inhibited the action of silane-containing porcelain primers and inhibited the chemical reaction between silane primer and glass ceramic.

In a recent study,[17] nuclear magnetic resonance analyses showed that the spectra peaks of 9.90 ppm (Si-O-Si- group) indicates the formation of silane

oligomers over time, which potentially impair the bonding performance. In this context, another study[51] showed that low Si-O-Si peaks were registered in SBU, probably due to increased propensity for intermediate reactions between silane and the variety of -OH sources in SBU (2-HEMA, MDP, VP-copolymer, water, etc). Thus, even if the silane in SBU does not help improve the adhesive properties, the mixture of all other components is likely to maintain a good chemical interaction of SBU with indirect substrates. This might be the reason why SBU showed better results than CFU in four of the five indirect substrates in our study, with the exception of LeGC.

Although MDP-containing universal adhesives showed higher mean μSBS after thermocycling when compared to MDP-free universal adhesives, there is no consensus on which is the better universal adhesive to be used in all substrates, as per the results of our study. Unfortunately, the exact amount of MDP is a manufacturer's trade secret. For instance, AMB, as well as SBU, showed higher mean μSBS even after thermocycling for four of the five indirect substrates, with the exception to LiDi. According to the manufacturer of AMB,[52] MDP has a higher reactivity, which results from redistributing the concentrations between solvents, water, and acidic monomers. Nevertheless, this concept has not been proven. In addition, the ideal amount of MDP to enhance its interaction with indirect materials is not consensual.

In fact, there are some studies that evaluated the effect of different concentrations of MDP on bonding to zirconia. While Yoshida and others[53] and Nagaoka and others[46] reported increasing bond strengths to zirconia for higher concentrations of MDP up to 1 wt%, Chen and others[54] showed that concentrations of MDP up to 10% showed higher bond strengths. However, the chemical affinity of MDP for zirconia is optimally achieved using 10 wt% MDP. On the other hand, Llerena-Icochea and others[55] evaluated experimental adhesives containing 3 to 15 wt% MDP when applied to zirconia. The results showed that there was no significant correlation between the concentration of MDP in the experimental adhesives and mean bond strengths to zirconia. Future studies need to be carried out to evaluate the exact concentration of MDP needed in the primers and universal adhesives to improve the bonding interaction to indirect resin composite, glass-matrix ceramics, and yttrium-stabilized zirconium dioxide.

Thus, the first null hypothesis was rejected, because all universal adhesives underwent a statistically significant bond strength reduction between the immediate time and after 10,000 thermal cycles, except AMB, FBU, and SBU for FeCe substrate. The second null hypothesis was rejected, as the mean microshear bond strengths among the different universal adhesives varied widely for each CAD/CAM material used.

CONCLUSION

Factors such as pH, type of solvent, and the presence of silane and/or MDP in the composition of each adhesive seem to be important in choosing a universal adhesive system for each particular substrate.

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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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Ceramic Inlay Bonded Interfaces in Minimally Invasive Preparations: Damage and Contributing Mechanisms in Sliding Contact

P Yu • Y Xiong • P Zhao • H Yu • D Arola • S Gao

It is recommended for clinicians to avoid placing centric occlusion on the bonded interface and to adjust a distributed occlusion contact to reduce the contact stress for inlay cavities with an unconventional tooth marginal angle or an axial wall located at the cusp inclination.

http://doi.org/10.2341/20-173-L

Evaluation of Novel Plant-derived Monomers-based Pretreatment on Bonding to Sound and Caries-affected Dentin

MVS Lemos • VG Araujo-Neto • D Lomonaco • SE Mazzetto • VP Feitosa • SL Santiago

Pretreatment with novel, plant-derived monomers is promising to reinforce the hybrid layer, since they preserved the resin–dentin bond strength and improved dentin bonding, especially to caries-affected dentin.

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Customized Fiber Post Improves the Bond Strength and Dentinal Penetrability of Resin Cementation System to Root Dentin

TP Leandrin • E Fernández • RO Lima • JF Besegato • WG Escalante-Otárola • MC Kuga

Customized fiber posts when placed with resin cements have the potential to improve bond strength and penetration of the adhesive into root dentin.

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Evaluation of Dentin Tubule Plugging Efficiencies and Effects on Dentin Surface Roughness of Dentin Desensitizing Agents, the Er,Cr:YSGG Laser, and Their Combination After Erosion-abrasion Cycles: An *In Vitro* Study

E Okur • GB Eyüboğlu

Combined laser–DDA treatments could be more effective than DDA treatments alone for dentin hypersensitivity (DH) treatment, particularly in challenging oral conditions, such as erosion and abrasion. These applications may help obtain longer-lasting and more satisfying results in the treatment of DH.

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Ceramic Inlay Bonded Interfaces in Minimally Invasive Preparations: Damage and Contributing Mechanisms in Sliding Contact

P Yu • Y Xiong • P Zhao • H Yu • D Arola • S Gao

Clinical Relevance

It is recommended for clinicians to avoid placing centric occlusion on the bonded interface and to adjust a distributed occlusion contact to reduce the contact stress for inlay cavities with an unconventional tooth marginal angle or an axial wall located at the cusp inclination.

SUMMARY

Background: In the preparation of inlay cavities, a choice must be made between conventional standard and minimally invasive preparation designs; in the long run, this choice can affect the integrity of the bonded interface.

Purpose: To evaluate the effect of minimally invasive cavity preparation designs on the extent and contributing mechanisms of damage to ceramic inlay bonded interfaces.

Methods and Materials: Tooth blocks with 90°, 120° and 75° marginal angles were prepared, representing tooth cavities with conventional

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standard and minimally invasive preparations with large divergence and convergence angles and bonded to monolithic ceramic (IPS e.max CAD). Vickers indentations were placed at various distances from the bonded interface. The indentation morphology and crack length were observed. Reciprocating wear tests were performed on the bonded interface with a 20-newton (N) vertical load. The wear depth and wear-scar morphology were characterized after increments of cyclic sliding contact.

Results: The 120° group exhibited longer indentation cracks in the ceramic, whereas the 75° group showed larger indentations in the enamel when compared to the 90° group (p<0.001). Consistent with the weaker edge crack resistance, the 120° group experienced the greatest wear (p=0.008), and the wear depth in the enamel of the 75° group exceeded that of the 90° group (p<0.001) in the early stage (5×102 cycles). However, no significant difference in the wear depth (p>0.147) and morphology were found at the later wear stage among the three groups.

Conclusion: Within the limitations of this study, minimally invasive preparations with 120° and 75° marginal angles can result in early sever damage at the ceramic inlay bonded interface but show comparable wear behaviors to the conventional 90° group at the later stage.

INTRODUCTION

Inlays are indirect restorations that are embedded within a tooth cavity to restore natural tooth morphology and function and are increasingly recommended by clinicians for minimally invasive treatment of tooth defects.[1] Among tooth-colored restorative materials, all-ceramic prostheses are preferred by doctors and patients due to better aesthetics, wear resistance, and chemical stability.[2]

The preparation of an inlay cavity is a necessary compromise to restore a damaged tooth. At the present time, various preparation guidelines exist for ceramic inlay restorations. According to the traditional preparation guideline, a divergence angle of approximately 6° to 10° between two internal axial walls is recommended in order to provide adequate retention force for restorations.[3-5] Nevertheless, with advancements in cement technology, the bond strength of present cement systems can exceed 50 megapascals (MPa),[6] no longer requiring a strict retention form for inlay restorations.[7,8] Alternatively, tooth preparation has come to be focused on maximal preservation of natural dental hard tissue, and a minimally invasive design should be considered for the given situation.[9-11]

After removal of the original filling or decayed tissue, the inlay tooth cavity might have a conventional divergence angle (Figure 1A2), an excessive divergence angle (Figure 1B2) or even a convergence angle (Figure 1C2). Accordingly, the marginal angle of the residual tooth structure could range from obtuse to acute. For cavities with a conventional divergence angle, the marginal angle of tooth tissue is also related to the location of the axial wall. When the axial wall is located at the central area of the occlusal surface, the marginal angle of the tooth tissue is about 90° (Figure 1A3). When the axial wall is located at the cusp inclination, however, the marginal angle is about 120° (Figure 1A3), due to the anatomic form of the tooth cusp.[3,4] In order to achieve a conventional divergent geometrical design in cavities with an excessive divergence angle or a convergence angle, following the traditional preparation guidelines would inevitably result in the removal of sound tooth tissue, especially the enamel near the occlusal surface of the cavity (Figure 1B3 and 1C3).[12,13] The removal of sound tooth tissue can be avoided in these situations by using a minimally invasive preparation. A cavity with a large divergence angle could be restored with an inlay restoration after a simple polishing treatment (Figure 1B4). However, the cement edge of the inlay restoration might be an acute angle, as this angle is determined by the beveled edge of the prepared tooth (Figure 2B2). For a tooth cavity with a convergence angle, a chamfer is not conducive to the production of models and prostheses. In this scenario, the proximal undercuts would be filled with resin composite or resin cement (Figure 1C4), resulting in a divergent geometrical form.[10,14] While the minimally invasive design might save more healthy tissue, it would also lead to a broader luting margin cervically (Figure 2C3).

The marginal angle of an inlay tooth cavity in the minimally invasive preparation design can be considered as falling into three categories in various situations: close to 90°, greater than 90°, or less than 90°. Considering the anatomic form of the tooth cusp and the parameter setting in previous studies,[4,10] marginal angles of 90°, 120° and 75° were selected for this study.

A requirement for long-term stability of an inlay restoration is an intact bonded interface.[15, 16] Destruction of interface integrity under chewing force is closely related to the occurrence of complications such as dentin sensitivity, marginal discoloration,

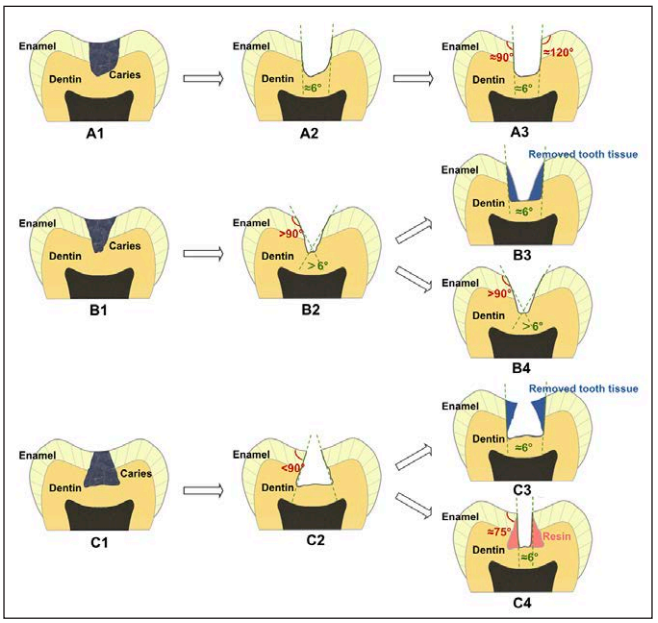

Figure 1. *Description of the experimental groups. (A1-C1): different types of decayed teeth; A2-C2: cavities remaining after removal of the original filling or decayed dental tissue; (B3, C3): inlay cavities in conventional standard preparation. (A3, B4, C4): inlay cavities in minimally invasive preparations.*

secondary caries, and prosthesis fracture.[17-20] A previous study indicated that minimally invasive preparations for mesial-occlusal-distal (MOD) inlays with undercuts show marginal adaptation equivalent to that of conventional preparation designs,[10] thereby serving as a preliminarily justification for their clinical application. Apart from internal adaptation, differences in marginal angle of the tooth tissue and ceramic inlay may influence the interface degradation process of inlay-restored teeth, because they lead to different stress distributions and marginal toughness at the bonded interface between tooth and restoration.[21-25] However, whether minimally invasive preparations will affect the stability of the bonded interface has not been reported.

The purpose of this study was to evaluate the effect of minimally invasive cavity preparation designs on the extent and contributing mechanisms of damage to bonded interfaces involving ceramic inlays by using reciprocating sliding wear tests. Due to the brittle nature of the materials, cracks may appear in the ceramic or enamel surface when stress intensity exceeds fracture toughness.[26,27] These cracks can undergo cyclic extension under the repetition of contact stress, and their intersection with each other can result in peeling and degradation of the bonded interface. Therefore, we introduced an indentation near the bonded interface to analyze crack behavior before analyzing damage under cyclic sliding contact. The null hypothesis was that the minimally invasive preparations would not affect the characteristics of the damage in bonded interfaces involving ceramic inlays.

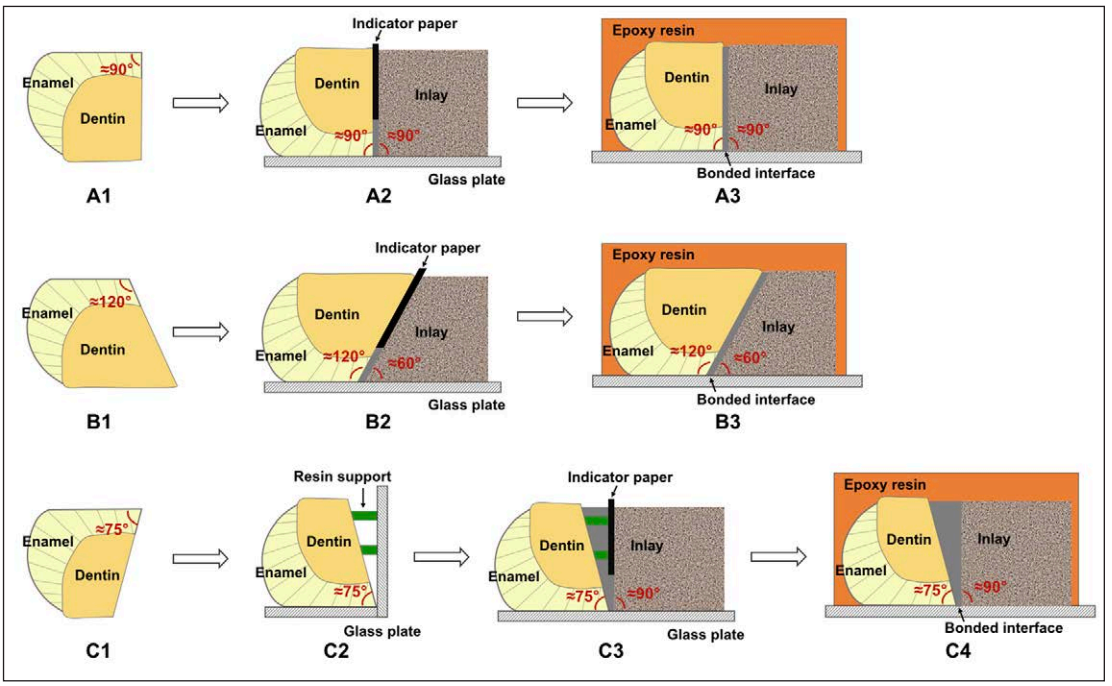

Figure 2. *Description of the specimen bonding procedures (section view). (A1-C1): tooth sections with flat occlusal surfaces; C2: tooth sections of 75° group with two resin supports; (A2, B2, C3): schematic diagram of the bonded specimens; (A3, B3, C4): the embedded specimens.*

METHODS AND MATERIALS

Specimen Preparation

The crowns of freshly extracted third molars (patient age: 18 years to 25 years) were cut into two sections along the mesial-distal axis with a slow diamond-abrasive slicing wheel (Struers Minitom, Struers) under continuous water coolant. The occlusal surfaces of tooth sections were ground flat with 280 grit silicon carbide (SiC) papers. A total of 90 tooth sections were assigned to three groups: the inner faces were ground to produce marginal angles of 90° (Figure 2A1), 120° (Figure 2B1), or 75° (Figure 2C1) and inspected with an angle ruler. Meanwhile, 90 leucite-reinforced ceramic sections (IPS e.max CAD, Ivoclar Vivadent, Schaan, Liechtenstein) of 6×6×4 mm^3 were cut and sintered according to the manufacturer's protocol. The cross sections of the ceramic sections that would serve as the bonding surface were ground with 280 grit SiC papers to produce marginal angles of 90° (Figure 2A2 and 2C3) or 60° (Figure 2B2). The permissible deviation of the marginal angle was ±3°.

After ultrasonic cleaning with deionized water (KQ-50B, Shumei, Kunshan, China), the abraded surfaces of the tooth sections were etched (30 seconds for enamel, 15 seconds for dentin) with 35% phosphoric acid gel (3M Oral Care, St. Paul, MN, USA), rinsed for 30 seconds with water spray, and dried with oil-free air. The abraded surfaces of ceramic sections were etched with 4.9% hydrofluoric acid gel (IPS ceramic etching gel, Ivoclar Vivadent) for 20 seconds, rinsed for 60 seconds, and air dried. Then the tooth sections with different marginal angles were bonded to the corresponding ceramic sections. For the tooth sections with a 75° marginal angle, a resin composite (Filtek Z350, 3M Oral Care) was applied incrementally on the dentin under the guidance of glass plates (Figure 2C2) to form two resin supports to facilitate the subsequent bonding between tooth and inlay. A dual-cured universal resin cement (Rely U200, 3M Oral Care) was mixed following the manufacturer's instructions and applied to the entire ceramic and tooth surfaces. The inner surfaces of the tooth and ceramic sections were pressed together with an axial load of approximately 0.5 kg, ensuring that the occlusal surface of the tooth section was in the same plane as the face of the ceramic section by using a glass plate (Figures 2A2, 2B2, and 2C3). To control the resin cement thickness, a single indicator paper with a thickness of approximately 50 μm was positioned at the periphery of the bonding surface near the dentin side. After removing excess cement, light curing was applied to each side of the specimen for 40 seconds with an LED-type light source (Bluephase, 800 mW/cm^2, Ivoclar Vivadent).

The bonded specimens were stored in artificial saliva for 24 hours at 37°C and then embedded (Figures 2A3, 2B3, and 2C4) in an auto-polymerizing acrylic resin (Struers). Then the bonded surfaces of all specimens were ground with SiC papers in a sequence of decreasing abrasive size (P280, P800, P1200, P2400, and P4000-grit) under water irrigation and highly polished with 3 and 0.04 μm abrasive particle solutions with felt cloths (Dac, Struers) on a dedicated instrument (Tegramin-30, Struers). Finally, the specimens were observed under an optical microscope (OM, BX51RF, Olympus, Tokyo, Japan) at 200×. The bonded interface remained intact and the measured interface width ranged from 30 μm to 80 μm.

Micro-Vickers Indentation Test

Micro-Vickers indentations were introduced on the enamel and ceramic portions of the polished specimens using a hardness tester (MVK-E, Akashi, Kanagawa, Japan) with 1 N load for 15 seconds in air, at distances of approximately 50, 100, 200, and 400 μm from the bonded interface edge to the center of the tip of the Vickers indenter (Figure 3). The diagonal of indentation was controlled to be parallel and perpendicular to the bonded interface. Ten specimens were chosen from each angle group, and eight indentations spaced at least 500 μm from each other were applied at four distances for each specimen. Then the specimens were observed under OM at magnification of 100×. The lengths of the two ends of the indentations were measured as indentation length (L1), and the longest lengths of the two ends of radial cracks running approximately parallel to the interface were measured as the indentation crack length (L2).

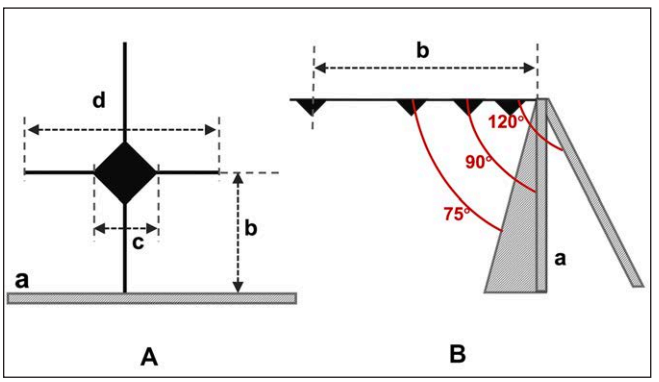

Figure 3. *Schematic diagram of the indentation test (A: vertical view, B: section view). Black diamond indicates the Micro-Vickers indentation; a: the bonded interface; b: the distance between indentation and bonded interface on occlusal surface (b=50, 100, 200, or 400 μm); c: the measured indentation length (L1); d: the measured indentation crack length (L2).*

Wear Tests

Reciprocating wear tests were conducted on the occlusal surface of specimens with ball-on-flat configuration by using a commercial tribometer (MFT-5000, Rtec-Instruments Inc, San Jose, CA, USA). Silicon nitride (SiN) balls with a diameter of 6.35 mm were used as the antagonists.[28,29] The tests were performed under simulated artificial saliva (A7990, Beijing Solarbio Science & Technology Co Ltd, China) lubrication at 25° room temperature. The testing parameters included a vertical load of 20 N, a sliding distance of 2 mm and a frequency of 1 Hz.[30,31] The occlusal contacts were marked with articulating paper to ensure that the displacement midpoint of the articulations was located at the bonded interface. A total of 5×10^4 cycles was performed, and three intervals of data analysis were employed after 5×10^2, 5×10^3 and 5×10^4 cycles. Eight tests were carried out for each cycle. Each wear scar was scanned using a white light interferometer (UP series, Rtec-Instruments Inc) operating in the vertical scanning mode equipped with the tribometer, and three-dimensional topography maps of the wear scars were reconstructed. Profiles of the wear scars were obtained from near the center of the scars and perpendicular to the bonded interfaces along the path of displacement. Taking the height of the unworn surface on both sides of the wear scar as a reference, the wear depths of the bonded interface area were calculated within the cement, the enamel, and the inlay material. For consistency, the locations of measurement for the enamel and inlay material were 30 μm from the bonded interface edge. Thereafter, representative specimens from each group were examined using scanning electron microscopy (SEM, INSPECTE, Czech Republic). The wear characteristics and mechanisms were analyzed from the wear-scar morphology.

Statistical Analysis

All data were analyzed with software IBM SPSS Statistics 20.0 (IBM, USA). A two-way analysis of variance (ANOVA) and Tukey multiple comparisons were used to compare the L1 and L2 measurements with different angles and distances from the bonded interface. A one-way ANOVA was performed to assess the differences in wear depth. The level of significance was defined at 0.05.

RESULTS

Indentation Crack Behavior

The results of L1 and L2 measurements are summarized in Figure 4. Representative micrographs of the indentations are shown in Figure 5.

On the tooth enamel surface, two-way ANOVA revealed that both L1 and L2 were significantly affected by the distance from the enamel/resin interface ($p<0.001$ for L1 and $p=0.001$ for L2) and the marginal angle ($p<0.001$ for L1 and $p=0.001$ for L2). Multiple comparisons among the means at the four distances showed that the L1 at

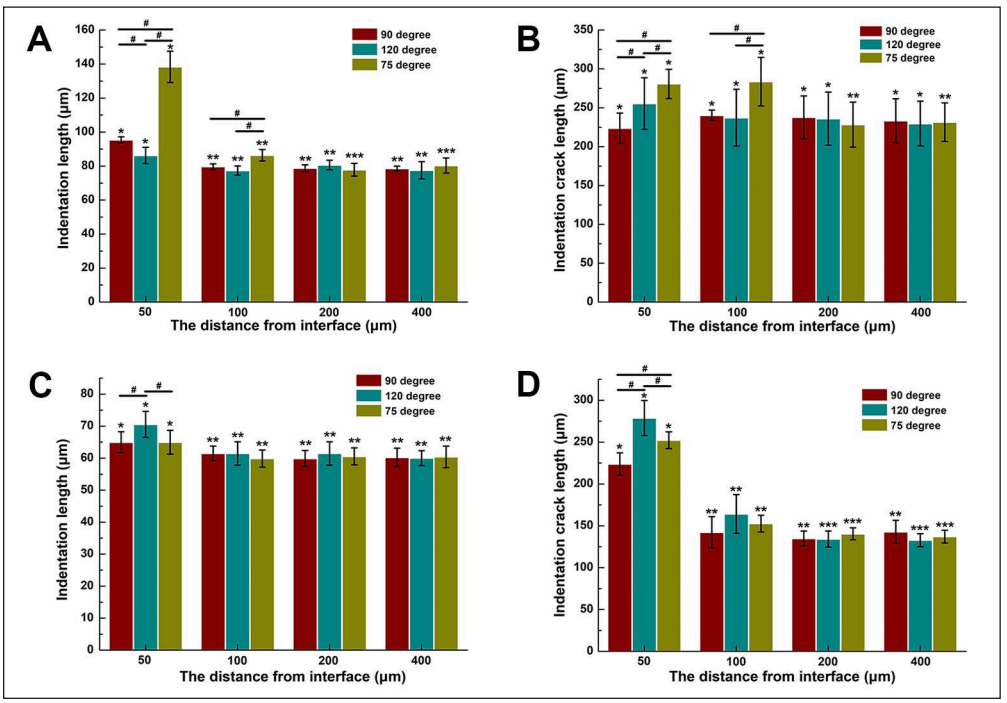

Figure 4. *Comparison of differences in indentation length (L1) and indentation crack length (L2) on tooth enamel (A, B) and ceramic (C, D) among the three groups. Same symbols (*,**,***) denote that there is no significant difference among different groups; # indicates there is significant difference between two groups at same measuring position; vertical error bars indicate the standard deviations.*

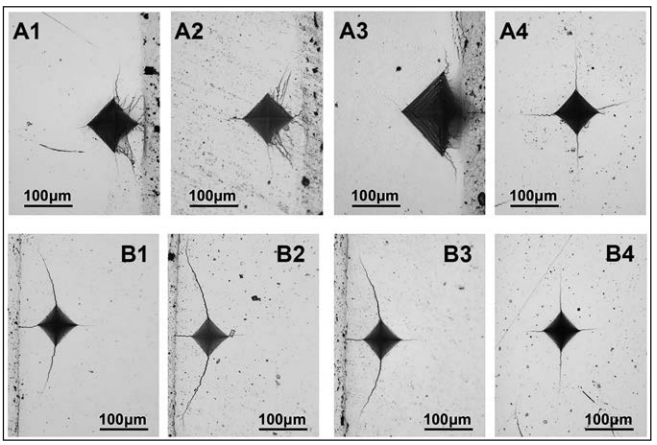

Figure 5. *Representative micrographs of the indentations and cracks in tooth enamel (A1-A4) and leucite-reinforced ceramic (B1-B4). (A1, B1): 50 μm-90° group; (A2, B2): 50 μm-120° group; (A3, B3): 50 μm-75° group; A4, (B4): 100 μm-90° group.*

a distance of 50 μm was the longest in each marginal angle group (p<0.001 for the 90° group, p=0.003 for the 120° group and p<0.001 for the 75° group). Among the three angles, the 75° group showed the biggest L1 and L2 (p<0.001) at the distance of 50 μm. The indentations for the 75° group were so large that they extended to the cement area, and the boundary of the cement area was not clear (Figure 5A3). In addition, the number of cracks was significantly greater (p<0.001) for the 50 μm distance. Besides radial cracks extending from the indentation corners, some lateral cracks were initiated from the indentation boundary, especially on the side flanking the cement (Figure 5A1).

On the ceramic surface, L1 and L2 were also affected by the distance from the enamel/resin interface (p<0.001 for L1 and L2) and the marginal angle (p=0.006 for L1 and p<0.001 for L2). Multiple comparisons indicated the largest L1 (p=0.008 for the 90° group, p<0.001 for the 120° group, and p=0.003 for 75° the group) and L2 (p<0.001 for all three groups) resulted from indentations placed 50 μm from the interface (Figures 4C and 4D). Among the three groups, the 120° group exhibited the largest L1 (p=0.002) and L2 (p<0.001). Radial cracks were initiated at the indentation corners and propagated parallel to the indentation diagonals and bonded interface within the ceramic. For the 50 μm indentation distance, the cracks gradually approached the interface with extension (Figure 5B1).

Wear Behavior

A comparison of typical vertical profiles and wear depths is presented in Figure 6. After 5×10^2 cycles, the vertical profiles exhibited a discontinuity in the vicinity of the bonded interface (Figure 6A1). Wear of the enamel exceeded that of the inlay regardless of the marginal angle. The 120° group experienced the greatest wear in the enamel (p=0.008), cement (p<0.001) and inlay material (p=0.010) among the three marginal angles (Figure 6A2), and the wear depth in the enamel of the 75° group exceeded that of the 90° group (p=0.038). At the next sliding contact interval (5×10^3 cycles), the wear scars appeared relatively smooth, with no abrupt discontinuities (Figure 6B1). There was no significant difference (Figure 6B2) in the extent of wear among the three groups (p=0.147 for enamel, p=0.249 for cement, and p=0.761 for ceramic) or among enamel, cement, and ceramic portions at the interface area (p=0.663 for the 90° group, p=0.359 for the 120° group, and p=0.288 for the 75° group). With further progression of the sliding contact (5×10^4 cycles), wear of the inlay increased rapidly and exceeded that of the cement and adjacent enamel (Figure 6C1). Interestingly, there was no significant difference (Figure 6C2) in the extent of wear in the bonded interface among the three marginal angle groups (p=0.659 for enamel, p=0.300 for cement, and p=0.178 for ceramic).

Wear Morphology

Micrographs documenting the morphology of typical wear scars after 5×10^2 and 5×10^4 cycles are shown in Figure 7 and Figure 8. After 5×10^2 cycles of sliding contact, the extent of damage in the bonded interface area was distinctly a function of marginal angle. The bonded interface of the 90° group exhibited the highest integrity overall. Only some small chips developed on the ceramic edge, and the enamel-cement interface appeared intact. For the 120° group, the width of the wear scar at the bonded interface was wider, suggesting a scuffing motion at the interface. Large cracks and exfoliation were evident on the ceramic edge (Figure 7B). Nevertheless, the interface between the cement and enamel still appeared to remain intact. For the 75° group, chipping and cracks were apparent both on the ceramic edge and in the adjacent tooth enamel (Figure 7C). The bond integrity between the enamel and cement appeared degraded as well.

With an increase to 5×10^3 cycles, the bonded interface appeared to be intact, with minimal evidence of damage, except for a few cracks concentrated in the ceramic just adjacent to the interface and not dependent upon the marginal angle (image not shown).

With an increase to 5×10^4 cycles, similar wear morphologies were found in the three groups. Although obvious cracks were identified in the ceramic near the bonded interface, the enamel-cement and cement-ceramic interfaces appeared intact (Figure 8).

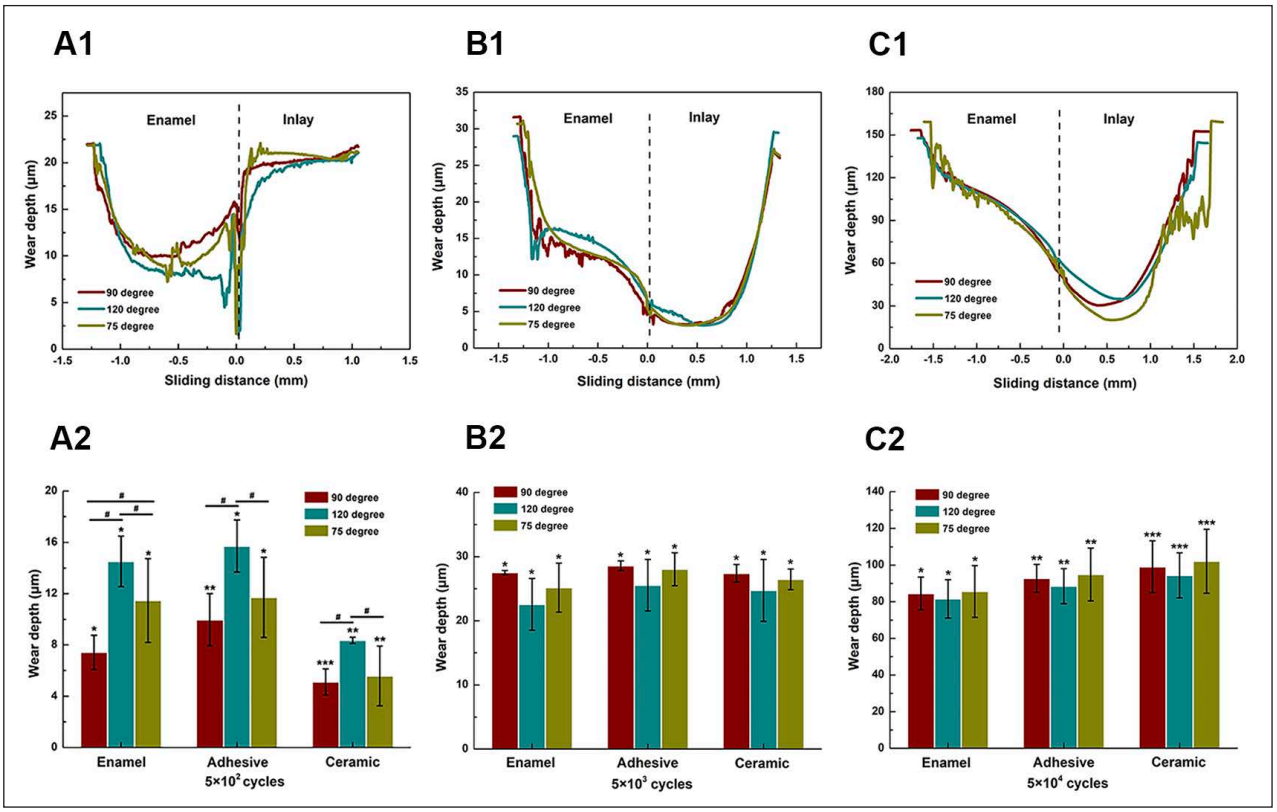

Figure 6. *Comparison of the typical vertical profiles and wear depths for the three marginal angle groups after different testing increments. (A1, A2): 5×102 cycles; (B1, B2): 5×103 cycles; (C1, C2): 5×104 cycles. Black dotted lines in A1-C1 indicate the bonded interface. Same symbols (*,**,***) denote that there is no significant difference in wear depth among tooth enamel, cement, and inlay material for each group; # indicates there is significant difference in wear depth between two groups; vertical error bars indicate the standard deviations.*

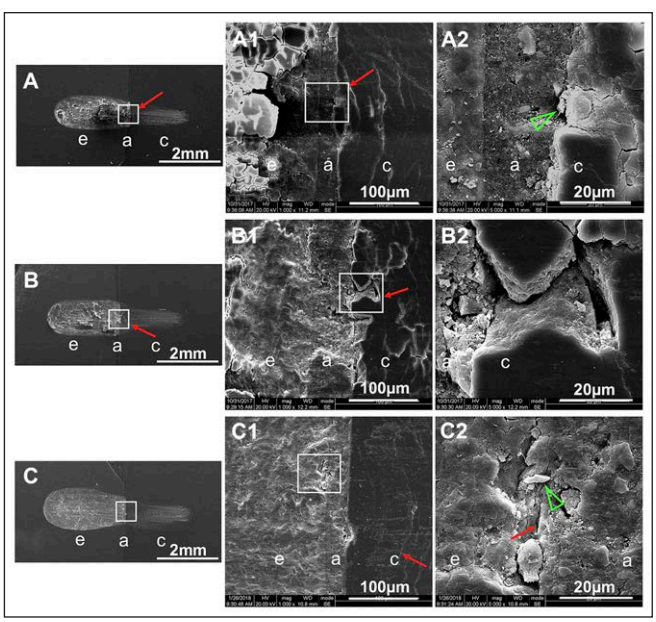

Figure 7. *Representative SEM micrographs of the worn surfaces after 5×102 cycles. (A): 90° group; (B): 120° group; (C): 75° group; white squares outline the bonded interface in wear scars and are shown in right images at higher magnification; e: enamel, a: cement, c: ceramic; arrows point to cracks; triangles indicate chipping.*

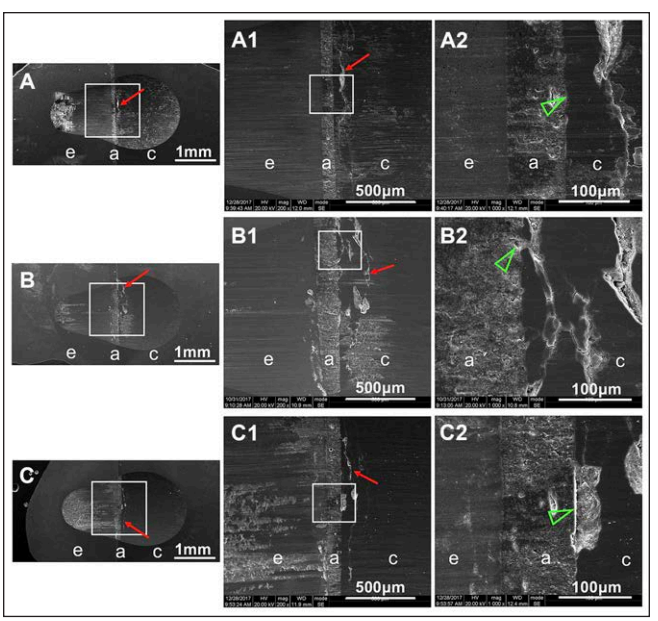

Figure 8. *Representative SEM micrographs of the worn surfaces after 5×104 cycles. (A): 90° group; (B): 120° group; (C): 75° group; white squares outline the bonded interface in wear scars and are shown in right images at higher magnification; e: enamel, a: cement, c: ceramic; arrows point to cracks; triangles indicate chipping.*

DISCUSSION

The present work studied the effect of cavity preparation design on the extent of wear and the damage mechanisms in ceramic inlay bonded interfaces. The results obtained showed that the wear depth and surface morphology varied as a function of the marginal angle at the initial stage of wear, while no significant difference was found in the later stages. Therefore, the null hypothesis was partially rejected.

In the indentation test, indentation cracks were dependent on the distance of the indentation from the interface, a finding in agreement with previous studies.[23,24] To some extent, the direction of crack extension reflects the weak area of the restoration. When the indentation was introduced near the bonded interface, more cracks were generated and/or the cracks were longer, thereby suggesting that the interface area is most vulnerable to damage induced by cyclic sliding contact. Similar to the results of the indentation damage test, the wear depth was most severe at the interface area and decreased with increasing distance from the interface. Therefore, to minimize marginal deterioration of inlay restorations, clinicians should be vigilant to avoid placing centric occlusion on the bonded interface if possible.

Apart from the importance of distance, the characteristics of cracks resulting from the indentations were also dependent on the tooth marginal angle. According to the work of Dejak and others,[21] the stress intensity factor for cracks increased and the critical stress causing fracture of the restoration decreased with a reduction in the edge angle of inlays. Similarly, Nishide and others[3] showed that when the ceramic marginal angle changed from 90° to 60° to 45°, the critical stress required for fracture decreased to 1/3 and 1/5 of the original values. Indeed, the L1 and L2 for the 120° group (with a 60° ceramic edge angle) near the bonded interface were significantly greater than those of 90° group and 75° group (both with 90° ceramic edge angle). At the early stage of sliding contact, the contact area between the bonded interface specimen and the antagonist ball was essentially a ball-on-flat configuration. As such, the contact stress was higher at the beginning of the cyclic wear testing. Numerous cone cracking or inner cone cracking modes appeared in the ceramic structures near the bonded interface, particularly in the 120° group, as also shown in the study of Zhang and others.[32] The cracks intersected with each other or combined with the more damaging radial crack that can initiate from either the top or bottom surface of the brittle materials, especially with the thinner ceramic on the edge, resulting in the formation of large irregular peeling fragments (Figure 7B). With the loss of ceramic support, the adjacent cement and enamel were exposed and underwent accelerated wear as a result of sliding contact.[3] This might be the cause of the significantly larger wear depth of the bonded interface of the 120° group compared to the others.

A bonded interface involves enamel supported by a foundation of dentin. When compared to enamel, dentin has lower hardness and elastic modulus and greater potential for stress relief.[27] When subjected to concentrated force, dentin can act as a damper to reduce the stress by viscous processes, which increases the resistance of enamel to brittle fracture. For the 75° group, the tooth margin had an acute angle, resulting in less enamel structure surrounding and supporting the indentation and a lack of the buffer action of inner dentin.[22] In this condition, the enamel surface collapsed under the indentation stress, resulting in a significantly larger indentation and numerous microcracks at the indentation boundary (Figure 5A3). Under cyclic sliding contact, small bundles of enamel rods underwent crushing, resulting in brittle fracture and chipping (Figure 7C). Without the support of the enamel, wear on the cement and the ceramic inlay were accelerated. Accordingly, the 75° group showed a larger wear depth than the 90° group at the initial stage of sliding contact.

Although both the 120° and 75° groups experienced more severe damage on the bonded interface area in the early stage of sliding contact, there was no significant difference in wear depth and damage characteristics at the later stages. This change in behavior with more prolonged contact could be the result of two factors. Firstly, the contact area between the wear scar and the antagonist ball increased, resulting in a decrease in contact stress. Secondly, due to its higher modulus, the ceramic inlay bears a larger portion of the contact load at the bonded interface area,[33,34] protecting the residual dental tissue and facilitating accelerated wear relative to the tooth enamel.[25] With continuation of the sliding contact, wear in the ceramic gradually exceeded that in the enamel, resulting in an uneven wear surface. Based on the history of the wear-scar morphology shown in Figure 9, the marginal angle of the dental tissue gradually increased and, with progression of the sliding contact, exceeded 90°. The ability to bear stress increases with increasing marginal angle.[21,22] Indeed, the SEM analysis also showed that there was no obvious damage in the nearby enamel at the later stage for the three groups. On the contrary, the marginal angle of the ceramic inlay decreased gradually with wear, resulting in a degradation of its load-bearing ability. As shown in Figure 8, all three groups exhibited obvious cracks and chipping of the ceramic adjacent to the bonded interface at the later stages of the evaluation.

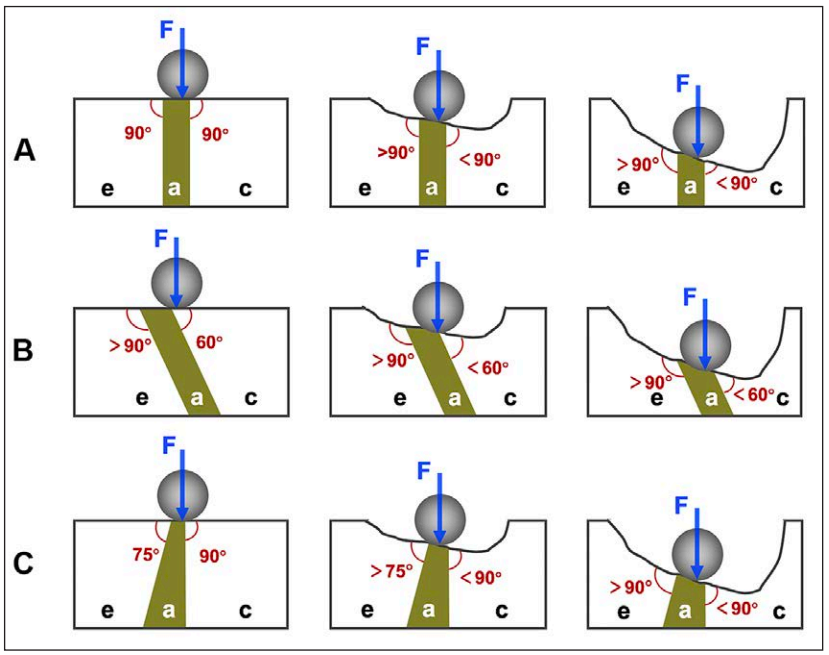

Figure 9. *Schematic representation of the evolution of wear at the ceramic inlay bonded interface with continuing sliding contact (number of cycles increases from left to right). (A): 90° group; (B): 120° group; (C): 75° group; e: enamel, a: cement, c: ceramic.*

Overall, results of the evaluation indicated that minimally invasive preparation designs with either larger or smaller marginal angles than conventional preparations experienced more serious interface damage in the early stages of function. However, no significant difference was found in wear damage behaviors at the later stage. Under very controlled conditions, the wear depth of bonded interface was about 20-30 μm after 5×10^3 cycles of sliding wear in this study, which is roughly equivalent to about 1 year of chewing movement in the mouth.[35] This means that the influence of tooth marginal angle on the integrity of the bonded interface might last less than 1 year. Of course, more serious interface damage in the early stage could facilitate the accumulation of plaque and pigment, increasing the risk of other complications.[36,37] Therefore, for inlay cavities with unconventional tooth marginal angles or axial walls located at the cusp inclination, the authors recommend that clinicians use a distributed occlusion contact in order to reduce contact stress and monitor the bonded interface area more frequently at the initial stage.

Although care was taken to study the effect of cavity preparation design on the extent of wear and damage mechanisms at bonded interfaces in a clinically relevant manner, it is important to recognize the differences between the test conditions and the clinic. Due to the complexity and uncontrollability of the oral environment, the bonded interfaces in this study were prepared with specific geometry and investigated under very controlled conditions, which could act as an important predictive tool for clinical performance.[38] However, the influence of variations in tooth anatomic form, pH, temperature, bacterial biofilm, and other challenges is relatively unknown. In addition, only specific tooth marginal angles were analyzed in this experiment, which was a preliminary investigation on the effect of tooth marginal angle on interface damage. The correlation between the marginal angle and the damage extent of the bonded interface and the critical angle affecting the damage behavior need to be further explored. Considering these limitations, further studies with more narrow grouping are currently underway to explore the importance of these additional factors.

CONCLUSIONS

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

1. Crack extension and wear damage within the vicinity of the bonded interface were significantly greater than those further away from the interface. The interface area is more vulnerable to damage induced by cyclic sliding contact.
2. Indentation crack resistance of the inlay bonded interfaces was significantly dependent on the marginal angle. In comparison to the 90° conventional preparations, the 120° group exhibited longer indentation cracks on the ceramic edge, whereas the 75° group showed significantly larger indentations on the enamel edge.
3. Under cyclic sliding contact, minimally invasive preparations with 120° and 75° marginal angles underwent more extensive damage at ceramic inlay

bonded interfaces in the initial stage of function but showed comparable damage behavior to the 90° group at the later stages.

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Regulatory Statement

The experimental procedure involving teeth was approved by the Research Ethics Committee of Sichuan University West China College of Stomatology (WCHSIRB-D-2016-046) and was in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Both the collection and use of teeth were performed with the informed consent of all the patients.

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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Evaluation of Novel Plant-derived Monomers-based Pretreatment on Bonding to Sound and Caries-affected Dentin

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Clinical Relevance

Pretreatment with novel, plant-derived monomers is promising to reinforce the hybrid layer, since they preserved the resin–dentin bond strength and improved dentin bonding, especially to caries-affected dentin.

SUMMARY

This study evaluated the influence of new monomers derived from cashew nut shell liquid (CNSL) applied for dentin biomodification on resin–dentin bond strength, nanoleakage, and micropermeability to sound and artificially-created caries-affected dentin. Human dentin specimens were assigned to five groups, according to the following dentin pretreatment solutions: Absolute ethanol (control), 2 wt% grape seed extract *(Vitis vinifera)*, 2 wt% cardol [from cashew nut shell liquid (CNSL)], 2 wt% cardol-methacrylate or 2 wt% cardanol-methacrylate applied on sound and artificial caries-affected dentin. Specimens were analyzed after 24 hour or 1 year of water storage. Microtensile bond strength (μTBS) (n=6), interface micropermeability (n=3), and silver nanoleakage (n=6) were assessed using a universal testing machine, confocal laser scanning microscope, and scanning electron microscope, respectively. In sound dentin, no difference in bond strength was observed between the groups in either storage period. In artificial caries-affected dentin, pretreatment with cardol-methacrylate resulted in statistically higher bond strength than all the other treatments in both storage periods. Cardol-methacrylate treatment resulted in less nanoleakage, along with improved interfacial integrity, compared to further treatments in artificial caries-affected

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dentin. Regarding micropermeability analysis, all treatments depicted deficient sealing ability when applied on artificial caries-affected dentin, with the presence of gaps in the control group. In conclusion, cardol-methacrylate is a promising plant-derived monomer to reinforce the hybrid layer, since it preserved resin–dentin bond strength and improved dentin bonding, especially to caries-affected dentin, a well-known harsh substrate for adhesion longevity.

INTRODUCTION

The resin–dentin interface, in particular, the hybrid layer formed after phosphoric acid etching and the use of etch-and-rinse adhesives, seems to be the vulnerable zone for resin composite restoratives.[1] Dentin bonds are prone to degradation, mainly by polymer hydrolysis and collagen breakdown accelerated by enzymes, such as matrix metalloproteinases (MMPs) and cysteine cathepsins (CTPs).[2-5]

Additionally, current minimally invasive dentistry determines the conservative removal of caries, keeping part of the tissue that is affected by caries in the cavity.[6,7] Adhesion to caries-affected dentin is challenges the creation of a uniform and homogeneous hybrid layer.[8] With the widespread popularity of minimally invasive dentistry, direct restorations are often bonded to caries-affected dentin, which is preserved due to its likelihood to remineralize.[9–11] Nevertheless, caries-affected dentin is porous, with areas of partially demineralized collagen that allow deeper demineralization during phosphoric acid etching. Indeed, with a thicker layer of etched dentin, the infiltration of monomers is compromised and more resin-sparse collagen fibrils are exposed to degradation.[8,12,13]

The use of MMP and CTP inhibitors has been demonstrated to be an alternative to improving dentin bond durability.[8,14] The recent strategy of dentin biomodification by using collagen cross-linkers[15] aims to increase the mechanical properties of the hybrid layer and unprotected collagen fibrils, thereby preventing interface degradation and providing long-lasting dentin bonds.[4,16,17] However, the use of synthetic agents, such as glutaraldehyde, can present great cytotoxicity.[18] Therefore, the demand for natural collagen cross-linkers has increased in recent years,[15,19,20] with proanthocyanidins (PACs) from grape seed extract (GSE) from *Vitis vinifera* showing improvements in dentin's ultimate tensile strength,[15] hardness,[21] elastic modulus,[22] and resistance against biodegradation.[23] However, a great disadvantage of PACs is the pigmentation of the dentin substrate.[24]

Other natural compounds, such as cashew nut shell liquid (CNSL), extracted from plants (*Anacardium occidentale* L), also have the potential for dentin biomodification, thanks to the long carbon chain (15 carbons) and terminal polyphenols, similar to PACs. The application of these substances as dentin pretreatments has been effective in promoting crosslinking and increasing the modulus of elasticity of demineralized collagen.[24,25] However, the long-term effects on dentin bonding, particularly, to caries-affected dentin, has not been studied to date.

Furthermore, the addition of methacrylate groups to the cardol and cardanol molecules allowed for the development of novel functional monomers that can interact both with the exposed collagen and with other methacrylate-based monomers present in the adhesive blend, enabling a reinforcement in bond strength and better durability of the interface.[26,27] This study is a pioneer in synthesizing and evaluating the effectiveness of cardol-methacrylate and cardanol-methacrylate on dentin adhesion.

Therefore, the aim of this investigation was to evaluate the influence of pretreatment using novel monomers derived from CNSL on dentin bonding, micropermeability, and silver nanoleakage. The two study hypotheses were: (1) There are no differences among the new monomers tested in terms of bond effectiveness (bond strength and nanoleakage) to sound and artificially created, caries-affected dentin over the two different storage periods, and (2) the sealing ability promoted by the adjunctive use of different biomodification agents is better than that of the control adhesive.

METHODS AND MATERIALS

Experimental Design

The factors investigated were: (1) Dentin pretreatment (five levels): absolute ethanol (negative control), 2 wt% GSE (*Vitis vinifera*, Mega-Natural Gold; Polyphenolics, Madera, USA), 2 wt% cardol (separated and purified from CNSL), 2 wt% cardol-methacrylate (synthesized from cardol), and 2 wt% cardanol-methacrylate (synthesized from purified cardanol extracted from CNSL). Two dentin substrates were assessed: sound dentin and artificially created caries-affected dentin. The micropermeability test was only performed after 24 hours; experiments were performed with two different storage periods—24 hours and 1 year. All reagents were diluted in EtOH/H_2O (1:1 volume ratio) with 5 minutes agitation at 25°C. The control group used absolute ethanol (≥99.8% ethanol, Sigma–Aldrich, St. Louis, MO, USA). The experiments undertaken were the microtensile bond strength (μTBS) test, dentin

micropermeability, and silver nanoleakage, with the latter two being qualitatively evaluated using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), respectively. Six bonded teeth were used in each group (n=6) for the μTBS and nanoleakage assessments, whereas, an additional three teeth per group (n=3) were prepared for the micropermeability evaluation.

Synthesis and Purification of New Monomers

Cardol and cardanol were obtained from industrial CNSL supplied by Amendoas do Brasil Ltda (Fortaleza, Brazil), separated by column chromatography (silica gel 60) and characterized by gas chromatography–mass spectroscopy.[28] The synthesis and purification of cardol-methacrylate and cardanol-methacrylate were undertaken according to the protocol of Ogliari and others,[27] by means of esterification of the phenolic compounds with methacryloyl chloride in order to attach the polymerizable methacrylate functionality. Synthesis of cardol-methacrylate and cardanol-methacrylate occurred by replacing a hydroxyl present in the aromatic ring with a methacrylate (Figure 1).

Preparation of Artificial Caries-affected Dentin

Sixty teeth were prepared from extracted human third molars stored in 0.1% thymol solution at 4°C for 1 month or less, or until use.

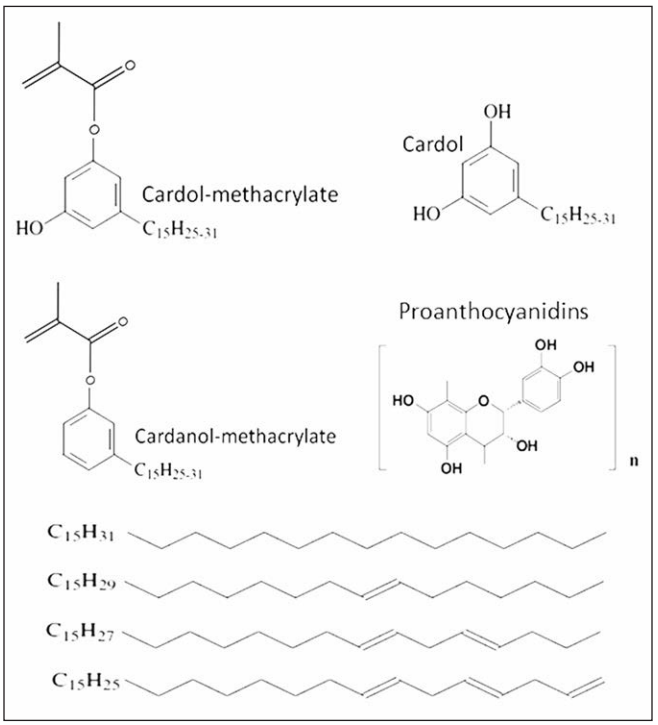

Figure 1. *Chemical structures of the biomodification agents tested.*

Each tooth was sectioned to expose a flat, middle dentin surface by using a slow-speed water-cooled diamond saw (Isomet 4000; Buehler, Lake Bluff, IL, USA), thereby removing the occlusal enamel crown and roots. Exposed dentin surfaces were ground using 320-grit SiC abrasive papers under constant water irrigation for 30 seconds to create a standardized smear layer.

Half of the specimens were subjected to pH cycling to create artificial caries-affected dentin. The occlusal dentin surface was polished with 1200-grit SiC paper to create a smooth surface. All further surfaces were protected with acid-resistant nail varnish. A layer of partially demineralized dentin approximately 200-μm thick[29] was created on the uncoated surface by pH cycling using the demineralizing solution containing 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50 mM acetic acid, and 5 mM NaN_3 adjusted to pH 4.8. The remineralizing solution consisted of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.13 M KCl, and 5 mM NaN_3 buffered to pH 7.0 with HEPES buffer. Each specimen was immersed in 10 mL demineralizing solution for 8 hours, followed by immersion in 10 mL of remineralizing solution for 16 hours, with fresh solutions used for each cycle. This procedure was performed for 14 days at room temperature.[29]

Microtensile Bond Strength Testing

All specimens (with sound dentin and caries-affected dentin) were etched using a 37% phosphoric acid gel (Condac 37%, FGM, Joinville, Brazil) for 15 seconds, followed by a copious water rinse for 30 seconds. The etched dentin surfaces were gently air-dried for 2 seconds to remove excess water. Each pretreatment (control, GSE, cardol, cardol-methacrylate, and cardanol-methacrylate) was actively applied for 60 seconds, followed by rinsing with distilled water for 30 seconds. The treated dentin was dried with absorbent paper to remove excess water, thus leaving a moist reflective surface. All specimens were bonded using the two-step etch-and-rinse adhesive Optibond Solo Plus (Kerr Corporation, Orange, CA, USA). The bonding agent was actively applied for 30 seconds, gently air-dried and light-cured for 20 seconds using the LED light-curing unit DB-685 (1100 mW/cm^2; Dabi Atlante, Ribeirao Preto, SP, Brazil). Five 1-mm-thick composite increments were built up (TPH Spectrum, Dentsply Caulk, Milford, DE, USA), each increment was light cured for 20 seconds.

All bonded teeth (n=6) were immersed in distilled water for 24 hours at 37°C and subsequently attached to an acrylic device with 90° rotation and sectioned with an Isomet saw to obtain sticks with cross-sectional areas

approximately 1.0 mm^2 (±0.04). The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo Corporation, Tokyo, Japan). Half of these sticks were tested immediately, and the remainder were stored in 3 mMol/L sodium azide solution for 1 year with exchanges performed every 15 days. The sticks were attached to a modified Geraldeli test apparatus[30] (Odeme Biotechnology; Joaçaba, SC, Brazil) with cyanoacrylate glue (Super Bonder Gel, Loctite, São Paulo, SP, Brazil) and tested to failure under tension in a universal testing machine (DL 2000; EMIC, Sao Jose dos Pinhais, PR, Brazil) with a crosshead speed of 0.5 mm/min. Bond strengths of the sticks from the same tooth were averaged, and the mean of each tooth was used as one statistical unit. A Shapiro–Wilk test was applied to all groups to analyze the normal distribution of errors, and the Bartlett test was used to determine homoscedasticity. After proving normal data, the results were statistically analyzed using two-way ANOVA (pretreatment and storage time) and Tukey test ($p<0.05$) individually for each dentin substrate.

Nanoleakage Evaluation

One stick from each restored tooth (n=6) was selected at random using Excel software (Excel 16.0, Microsoft Corporation, Redmond, WA, USA) for the nanoleakage test (n=6). The sticks were collected before microtensile testing. The nanoleakage test was performed, as previously described by Tay and others,[31] by using 50 wt% ammoniacal silver nitrate solution. The specimens were immersed in the tracer solution for 24 hours and then immersed in a photo-developing solution for 8 hours under a fluorescent light to reduce the silver ions into metallic silver grains. Thereafter, the specimens were rinsed with distilled water, embedded in epoxy resin stubs, and polished using successive 600-, 1200-, and 2000-grit wet SiC papers and 1-μm diamond paste (Buehler). The specimens were cleaned for 5 minutes in an ultrasonic bath after each abrasive/polishing step. The specimens were dehydrated in silica gel for 24 hours, coated with carbon, and examined using a field-emission SEM (Quanta FEG 450, FEI, Amsterdam, Netherlands) in the backscattered electron mode with 1000× and 3000× standardized magnifications.

Micropermeability Characterization

To perform the micropermeability test, three additional teeth per group were restored (n=3). The teeth were bonded, as previously described, with the adhesive resin doped with 0.1 wt% rhodamine-B (Sigma–Aldrich) and assessed using CLSM, according to a previously published protocol.[32] The micropermeability of the resin–dentin interfaces was evaluated using a 0.3 wt% aqueous fluorescein (Sigma–Aldrich) solution. This dye was perfused for 3 hours under 15 cm H_2O simulated pulpal pressure to test the sealing ability of the adhesive after different pretreatments.[32] The specimens were subsequently cut into 1-mm-thick slabs, slightly polished with 2000-grit polishing paper, and sonicated for 2 minutes. All steps were performed in environments with minimal light at room temperature (25°C).

The specimens were evaluated immediately after cutting using a CLSM instrument (LSM 710; Carl Zeiss, Munich, Germany) equipped with a 63× 1.4 NA oil immersion lens by using 488-nm and 568-nm laser illumination. CLSM fluorescence images were acquired with a 1-μm z-step to optically section the specimens up to 20 μm below the surface. The z-stack scans were compiled into single projections. Each resin–dentin interface was entirely characterized, and the images were captured along the bonded interfaces, representing the micropermeability characteristic from each group.

RESULTS

The means and standard deviations of μTBS on sound dentin are presented in Table 1. No difference was observed between the groups in this substrate after both storage periods ($p>0.05$). However, the control group did not maintain bond strength after 1 year ($p<0.001$). All experimental biomodification agents kept the resin–dentin bond strength stable after 1-year aging ($p>0.05$).

The results of μTBS on caries-affected dentin are presented in Table 2. Pretreatment with cardol-methacrylate resulted in significantly higher bond strengths than all other pretreatments in both the storage periods. In the immediate period, cardol and GSE presented statistically significant higher bond strength than the control group ($p<0.05$). However, no difference was observed between cardanol-methacrylate and the control groups ($p>0.05$). In addition, the resin–dentin bond strength was preserved after 1-year aging with pretreatments using cardol ($p=0.325$) and cardol-methacrylate ($p=0.103$).

Representative images of silver-infiltrated specimens of sound and caries-affected dentin substrates are illustrated in Figures 2 and 3, respectively. All biomodification agents on sound dentin resulted in great reduction of silver impregnation when compared to the control group in a 24 hour period (Figure 2), except cardanol-methacrylate (Figure 2-B1), which resulted in more nanoleakage even reaching the adhesive layer (Figure 2-B3). After 1-year aging, the cardol-methacrylate and GSE groups presented less silver infiltration (Figures 2-D2 and E2).

Table 1: *Mean (SD) Microtensile Bond Strengths in MPa for Sound Dentin*[a]

Sound Dentin	**Storage Period**[b]	
	24 Hour	**1 Year**
Control	48.6 (7.9) [47] Aa	26.1 (6.1) [41] Ab
Cardanol-MA	45.6 (3.5) [55] Aa	38.3 (5.7) [46] Aa
Cardol	42.5 (2.7) [49] Aa	36.5 (5.4) [40] Aa
Cardol-MA	36.2 (8.1) [58] Aa	36.9 (8.5) [50] Aa
GSE	37.1 (10.1) [54] Aa	36.2 (7.9) [42] Aa

Abbreviations: GSE, grape seed extract; MA, methacrylate.

[a] *Mean values with the same uppercase letters (column) and lowercase letters (row) are not significantly different (p>0.05).*

[b] *Numbers in brackets give the number of sticks tested per group in each period.*

Table 2: *Mean (SD) Microtensile Bond Strength in MPa for Artificially created Caries-affected Dentin*[a]

Caries-affected Dentin	**Storage Period**[b]	
	24 Hour	**1 Year**
Control	9.2 (7.2) [39] Ca	6.2 (0.7) [29] Cb
Cardanol-MA	10.0 (3.3) [41] Ca	5.7 (2.9) [32] Cb
Cardol	19.6 (5.1) [48] Ba	16.6 (6.8) [37] Ba
Cardol-MA	33.5 (7.3) [54] Aa	28.3 (8.9) [43] Aa
GSE	21.3 (3.8) [42] Ba	7.0 (3.1) [35] Cb

Abbreviations: GSE, grape seed extract; MA, methacrylate.

[a] *Mean values with the same uppercase letters (column) and lowercase letters (row) are not significantly different (p>0.05).*

[b] *Numbers in brackets give the number of sticks tested per group in each period.*

With caries-affected dentin (Figure 3), the porous zone of partially demineralized dentin 5-50 μm beneath the hybrid layer was fully infiltrated by silver deposits (Figures 3-A1 and E1). The presence of cracks and gaps was often noted in the control, cardanol-methacrylate, and GSE interfaces (Figures 3-A2, -B1, and -E2, respectively). Cardol-methacrylate pretreatment yielded less nanoleakage and better interface integrity than all further pretreatments (Figure 3-D). In cardol-methacrylate (Figure 3-D1) and GSE (Figure 3-E1) interfaces, a silver-free separation region between the hybrid layer and porous caries-affected dentin was observed, demonstrating some protection of dentin collagen. This same zone was noted in the cardol-methacrylate group after 1 year of aging (Figures 3- D2 and D3).

On the micropermeability analysis, all treatments showed deficient sealing ability when applied on caries-affected dentin, with the presence of interfacial gaps in the control group (Figure 4-A2). More fluorescein uptake was observed in the hybrid layers created using cardanol-methacrylate (Figure 4-B2) and GSE pretreatments (Figure 4-E2). In sound dentin, the control group showed intense fluorescein uptake in the hybrid layer, thereby indicating high micropermeability, while the pretreatment using cardol (Figure 4-C1) and cardol-methacrylate (Figure 4-D1) attained improved dentin sealing ability.

DISCUSSION

The development of new monomers derived from cardol and cardanol could be attributed to the proven ability of these molecules to improve the mechanical properties of collagen.[24] These molecules are extracted from CNSL—a natural renewable source obtained from the cashew industry—and exhibit three important advantages. (1) They are easy to obtain, since several thousands of tons of CNSL are produced yearly by cashew nut companies, which results in low production costs. (2) Finding various applications of CNSL and its compounds has environmental benefits, since industrial CNSL exhibits slow biodegradation and

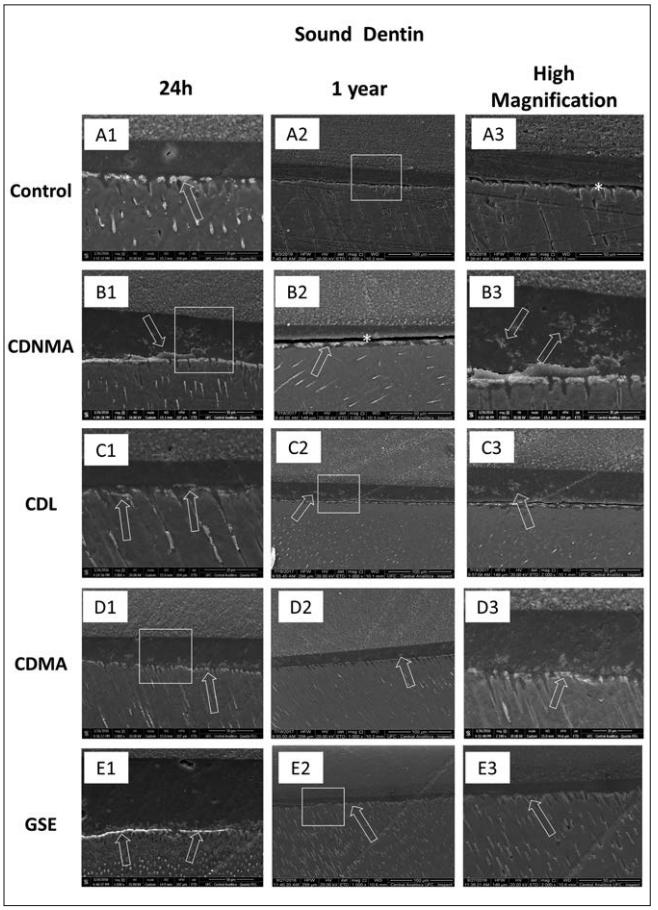

Figure 2. *Scanning electron microscopy (SEM) micrographs of resin–dentin interfaces illustrating the most common nanoleakage characteristics observed after silver uptake. Open arrows indicate silver deposits. The asterisks indicate gaps. CDL, cardol; CDMA, cardol-methacrylate; CDNMA, cardanolmethacrylate; GSE, grape seed extract.*

cannot be discarded in the environment. Moreover, large concentrations of cardol and cardanol can be obtained from CNSL after processing cashew nuts at high temperatures. (3) Cardol and cardanol are noncytotoxic compounds in low concentrations.[33,34] CNSL contains 60%-75% cardanol and 15%-20% cardol. Cardanol has been widely studied and has various chemical applications in the polymer, mineral oil, and ferrofluid industries.[28,35,36] Recently, a new polymer derived from cardanol has been demonstrated to be effective for tubular occlusion and possible treatment of dentin sensitivity.[37] Conversely, cardol has been poorly studied.

The presence of the methacrylate group in cardol-methacrylate and cardanol-methacrylate molecules allows for an interaction between these new monomers with monomers present in the formulation of bonding agents (e.g., triethylene glycol dimethacrylate and 2-hydroxyethyl methacrylate). However, before the copolymerization reaction, the new monomers are applied as a pretreatment in order to produce a chemical reaction with dentin collagen.[24] In fact, these reactions, promoted by cardol-methacrylate, increased the dentin bonding properties as observed in present outcomes (Tables 1 and 2), especially in caries-affected dentin, which has more exposed collagen. Pretreatment using cardol-methacrylate showed the best overall results with respect to bond strength and silver nanoleakage at both the substrate evaluations. Therefore, the first hypothesis was rejected.

According to Moreira and others,[24] the interaction between cardol/cardanol and collagen occurs by two mechanisms: first, forming hydrogen bonds by the presence of phenolic hydroxyl and, second, the hydrophobic bonds promoted by the long carbon chain. On the other hand, grape seed extract is unlikely to induce the formation of hydrophobic interactions.

Cardanol-methacrylate has a very similar chemical structure to cardol-methacrylate, but without the phenolic hydroxyl functionality attached to the aromatic ring. According to reports in the literature, the presence of the hydroxyl group attached to the aromatic ring allows strong interaction with collagen fibrils.[16,38] Therefore, the lack of this group allows only a hydrophobic interaction between cardanol-methacrylate and collagen fibrils. The comparison between the results obtained with cardol-methacrylate and cardanol-methacrylate indicate that the absence of a hydrophilic phenolic hydroxyl group (–OH) seems to compromise the penetration of cardanol-methacrylate in the water-rich demineralized collagen network. The lack of hydroxyl functionalities in cardanol-methacrylate, unlike in cardol-methacrylate, is an adequate explanation for its lower bond strength in caries-affected dentin. In addition, a remarkable difference was observed in silver nanoleakage between these two monomers (Figures 2 and 3).

Although the microtensile results were statistically lower than those observed with cardol-methacrylate, the cardol pretreated group showed stability of bond strength in dentin affected by caries, which indicates a potential effect of forming stable crosslinks, as previously demonstrated in the literature.[20] The integrity of the hybrid layer after storage represents a more relevant clinical outcome than high values of bond strength[39]; therefore, the results of both substances are promising for use in adhesive dentistry.

Nanoleakage images acquired at 24 hours in caries-affected dentin showed an intense concentration of silver deposits a few micrometers below the hybrid layer (Figure 3). This occurs due to caries-affected dentin

having areas of organized but partially demineralized collagen,[40] but this zone is closer to the adhesive layer in the control group than in the GSE group. Silver impregnation was not observed in the groups pretreated with cardol-methacrylate (Figure 3-D1), suggesting the crosslinking of collagen by means of hydrophobic interactions (through long carbon chains) was effective. After 1-year aging, the presence of silver near the hybrid layer was observed in all the experimental groups. However, the cardol-methacrylate application showed no silver infiltration into the hybrid layer (Figures 3-D2 and D3). Furthermore, little infiltration of silver after 1 year revealed a permanent protective effect on collagen, which seems to explain the favorable results of bond strength in caries-affected dentin (Table 2).

The control group showed a large infiltration of silver in both the substrates and the storage times; this is probably due to the incomplete penetration of monomers in the layer of demineralized collagen and

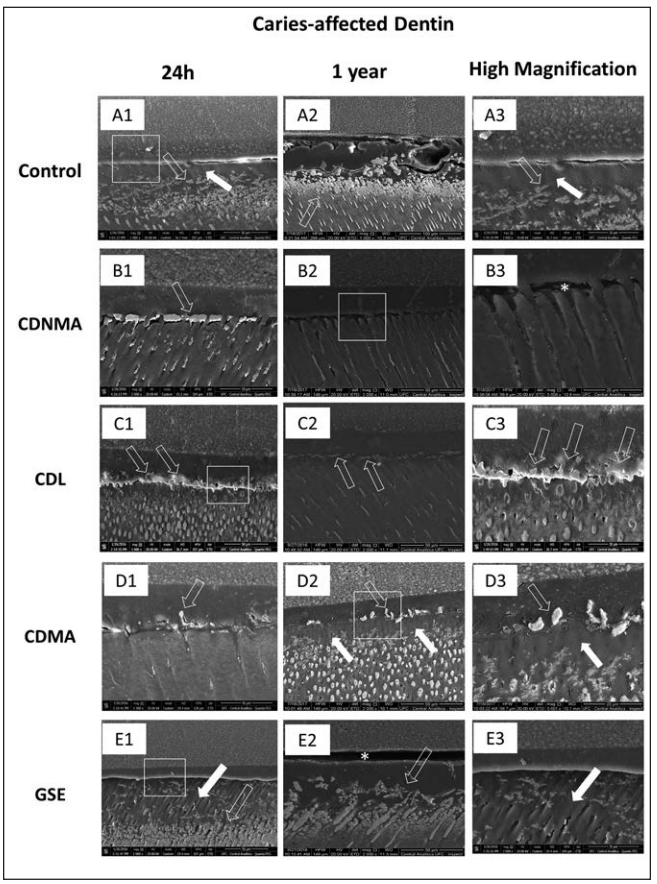

Figure 3. *Scanning electron microscopy (SEM) micrographs of resin–dentin interfaces illustrating the most common nanoleakage characteristics observed after silver uptake. Open arrows indicate silver deposits. White arrows indicate the protective zone between the hybrid layer and silver deposits. The asterisks indicate gaps. CDL, cardol; CDMA, cardol-methacrylate; CDNMA, cardanolmethacrylate; GSE, grape seed extract.*

the action of collagenolytic enzymes.[39,41] The structural reinforcement of collagen with the use of crosslinking agents and the use of amphipathic monomers, such as cardol-methacrylate and cardanol-methacrylate, could reduce these effects.

In caries-affected dentin, the decrease in bond strength and the increase in the amount of silver in the GSE group after 1 year seems to have occurred due to the reversibility of the bonds formed, which can be broken through hydrolysis, since these molecules are polar.[25,42] This outcome has been corroborated with other studies in the literature that also observed this effect.[24,25,43]

The micropermeability outcomes (Figure 4) indicated intense dye accumulation in the control interfaces for both the substrates. Dye penetration at the composite–dentin interface indicates incomplete adhesive infiltration into dentin, thereby resulting in a deeply demineralized dentin zone with exposed collagen fibrils.[44] Nevertheless, with experimental biomodification agents, the presence of fluorescein was notably reduced. This can be explained by the occupation of intrafibrillar and interfibrillar spaces by the plant-derived compounds, which were able to induce collagen cross-linking. The reduced uptake of fluorescein near the hybrid layer observed in the experimental groups indicates better sealing ability. Therefore, the second hypothesis should be accepted.

Indeed, the optimal outcomes of bond strength attained by using cardol-methacrylate with both sound and caries-affected dentin highlight such a monomer as a promising agent for dentin biomodification. However, one limitation of the present investigation and research design comprises the use of a simplified artificial caries model. Therefore, more studies are needed to evaluate other properties of this novel monomer in situations closer to clinical reality, its effects on resin polymerization, MMP/CTP inhibition capacity, cytotoxicity, and its use with self-etching adhesives.

CONCLUSIONS

Cardol-methacrylate is a promising monomer when used as an etch-and-rinse adhesive pretreatment. It might be used to reinforce the hybrid layer, as it preserves resin–dentin bond strength in both sound and caries-affected dentin; it also improves dentin sealing ability.

Acknowledgments

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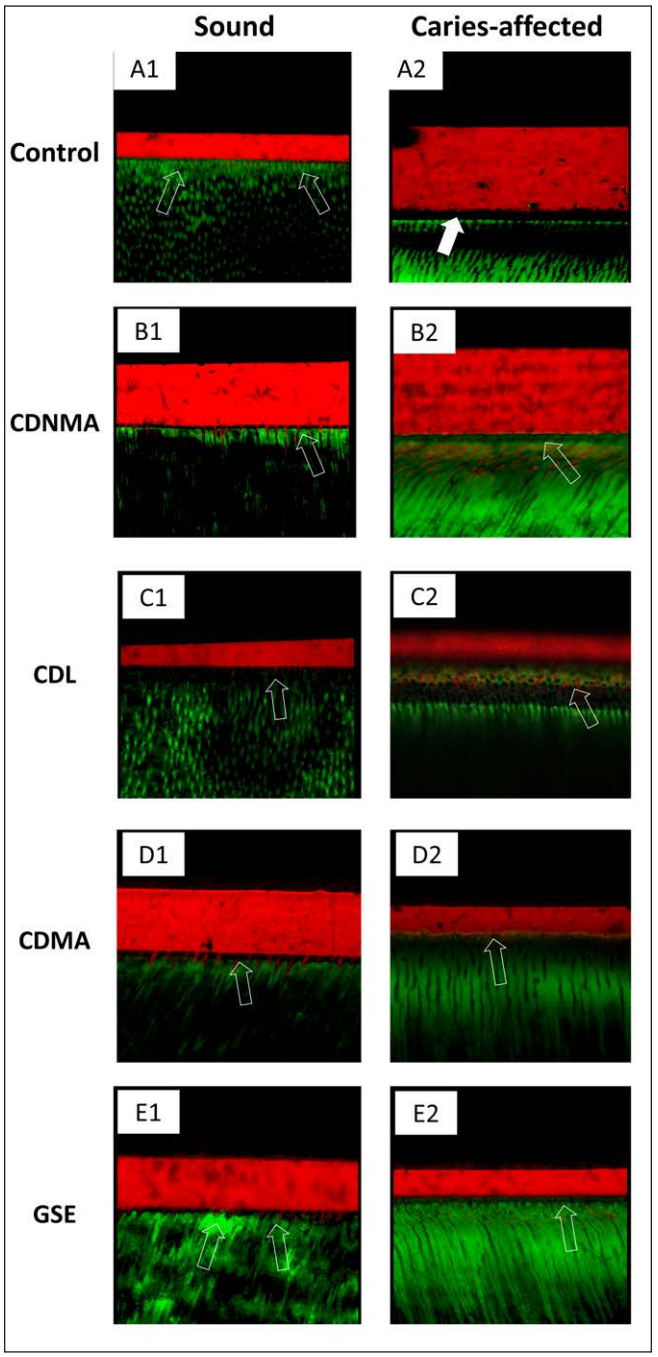

Figure 4. *Confocal micrographs showing the main features of fluorescein micropermeability. Open arrows indicate fluorescein infiltration at and near the hybrid layer. White arrow in A2 indicates a gap. HL in D3 indicates hybrid layer. CDL, cardol; CDMA, cardol-methacrylate; CDNMA, cardanolmethacrylate; GSE, grape seed extract.*

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies of the Research Ethics Committee on Investigations Involving Human Subjects of Federal University of Ceara. The approval code issued for this study is 1482602.

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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Customized Fiber Post Improves the Bond Strength and Dentinal Penetrability of Resin Cementation System to Root Dentin

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Clinical Relevance:

Customized fiber posts when placed with resin cements have the potential to improve bond strength and penetration of the adhesive into root dentin.

SUMMARY

Objective: This study aimed to evaluate the effect of fiber post customization on the bond strength (24 hours and 6 months), resin cement thickness, and dentinal penetrability of Adper Scotchbond Multi-Purpose – RelyX ARC (AS-RA), RelyX U200 (R2), and Scotchbond Universal – RelyX Ultimate (SU-RU) cementation systems to root dentin from the cervical-, middle-, and apical-thirds of the post space.

Methods: One hundred twenty bovine incisors were endodontically treated. After post space preparation, the roots were divided into six groups, according to the luting protocols (AS–RA, R2, SU–RU) and the type of fiber post [noncustomized post (NC) and customized post (C)]. Customization procedures were peformed using a resin composite (Z350 XT). 24 hours (n=60) or 6 months later (n=60), specimens from the cervical-, middle-, and apical-thirds of the post space were submitted to cementation system thickness measurement, bond

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strength evaluation, and dentinal penetrability analysis with Confocal Laser Scanning Microscopy (CLSM). Failure mode was classified as adhesive, cohesive, or mixed. Data were submitted to ANOVA and Tukey tests (α=0.05).

Results: Cementation protocols with customized fiber posts presented the lowest cementation system thickness, regardless of the cementation system or post space-third (p<0.05), and the highest bond strength values (p<0.05), regardless of the third space (p>0.05), for both periods (24 hours or 6 months). The comparison of push-out bond strength values between 24 hours and 6 months showed a reduction in all groups for the cervical-third (p<0.05). For the middle-third, only noncustomized groups showed reduction (p<0.05). For the apical-third, no reduction was observed (p>0.05).

Conclusions: Anatomical customization favored both the bond strength of cements to dentin and the dentinal penetrability, but with lower cementation system thickness, regardless of cement composition and adhesive strategy.

INTRODUCTION

Glass fiber posts are used as an alternative treatment to replace metallic posts in endodontically treated teeth. Their modulus of elasticity is similar to root dentin and resin cements, which promotes more homogeneity of stress distribution in the root and less probability of root fracture. Furthermore, glass fiber posts are more esthetic and avoid dental discoloration by corrosion products from the metallic alloys.[1-3]

However, the post morphology is different from the root canal anatomy. This difference may increase the cement thickness because of poor spatial relationship between them,[4] leading to increased shrinkage and a higher chance of axial displacement due to poor adaptation of the post. As a result, reduction in both degree of conversion and bond strength of the cementation system to root dentin can be observed.[5,6] Therefore, customized posts have been proposed to minimize these effects, since customization using resin composite may improve the post adaptation into the root canal as well as reduce the cement thickness.[7,8]

Furthermore, the type of cementation system may influence the clinical success of the fiber posts in endodontically treated teeth.[2,9,10] Conventional resin cements with etch-and-rinse, self-etching, and self-adhesive systems are the most used cementation systems in luting procedures.[11,12]

Three-step etch-and-rinse adhesive systems (eg, Adper Scotchbond Multi-Purpose) are some of the most effective dentin adhesives.[13] However, their clinical steps must be carefully performed, otherwise, failure may negatively affect the adhesive interface.[14] Moreover, several resin cements have been used with these types of adhesive systems; thus, whether any adhesive failure occurs, the post retention and its clinical longevity may be compromised.[2,13]

Self-adhesive cementation systems have been recommended to minimize the challenges of etch-and-rinse systems.[15] Their adhesion mechanism is related to the acid phosphate monomers that chemically bond to hydroxyapatite from the dentin substrate[16,17]; therefore, the application of adhesive systems is not required during the bonding procedure.[15] On the other hand, the adhesive efficiency is directly related to the clinical conditions of the dentin surface, such as presence of residues and type of endodontic treatment that was previously performed.[16]

Universal adhesive systems combined to conventional resin cements presents better interaction with dentin than self-adhesive cements, since not remove all of the smear layer is removed at adhesive interface, which reduces the common bonding errors associated with etch-and-rinse adhesives systems.[18]

Mechanical retention favors the action of cements and, consequently, improves the post's retention and stability.[7,19,20] Customized fiber posts have been recommended to be used with direct resin composites. By a simple technique, the post customization involves the use of resin composites to fill the space between the post and the root canal.[8] Thus, customization can reduce the cement thickness, which improves the post adaptation into the root canal and the friction retention to the dentin surface.[7,21,22] Although the frictional retention is crucial to the post stability inside the root canal,[19,20] other factors, such as, the anatomy of the root canal, endodontic treatment strategy, and luting system may also affect the stability.[13,15,23-25]

Many questions are still unclear regarding the interaction between the type of cementation system and the post customization on the adhesive interface, and which luting system should be used with customized fiber posts and vice versa.

Therefore, the aim of this study was to evaluate the effects of customized posts on the bond strength, dentinal penetrability, and adhesive failure mode of conventional cementation systems (Adper Scotchbond Multi-Purpose associated with RelyX ARC and Scotchbond Universal with RelyX Ultimate), and self-adhesive (RelyX U200), in the dentin at cervical-, middle-, and apical-thirds of the post space. Push-out testing and confocal laser scanning microscopy (CLSM) to evaluate the bond strength and

dentinal penetrability were, respectively, used. The null hypotheses tested were that the customized post does not influence the bond strength or dentinal penetrability of different cementation systems.

METHODS AND MATERIALS

Sixty bovine incisors with similar anatomy and dimension were stored in 0.1% thymol solution (pH 7.0) at 4°C ± 1°C until use.

Post Space Preparation

The root length was standardized at 15 mm length from the root apex. A #15K file (Denstply Maillefer, Petrópolis, RJ, Brazil) was introduced in the root canal until it was visible at the radicular apex. Then, the foraminal opening was sealed with cyanoacrylate resin (Super Bonder; Loctite, São Paulo, SP, Brazil). The root canals were prepared using ProTaper Rotary System technique up to the F5 instrument (Maillefer, Ballaigues, Switzerland) according to the manufacturer's recommendations. Then, irrigation was performed using 5 mL of 2.5% sodium hypochlorite solution (Asfer, São Caetano do Sul, SP, Brazil) at each instrument change.

Final irrigation was performed with 3 mL of 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) for 3 minutes and 5 mL of 2.5% NaOCl. The root canals were dried using absorbent paper points, obturated with epoxy resin-based sealer (AH Plus, Dentsply) and F5 gutta-percha master cone (ProTaper, Dentsply).[26] After that the roots were kept at 37°C ± 1°C for 7 days.

The intracanal preparation was performed using a #2 bur (White Post DC System, FGM, Joinville, SC, Brazil) at 11-mm length. Afterwards, the root canal was irrigated using 10 mL of distilled water and dried using absorbent paper points.

Sample Size

In this study, bond strength evaluation was considered the main outcome, with a calculated sample size (n=20), considering the comparison between two reported similar fiber post groups[7] [effect size=0.86, power 1-B (0.85), alpha (0.05)].

Experimental Groups

The fiber post surface was cleansed using 95% ethanol (Rinse-N-Dry, Racine, WI, USA). Then, silane (Prosil, FGM) was applied throughout its length. Since this study aimed to evaluate the effects of the customization of fiber posts, noncustomized or customized posts cemented with RA, R2, or RU cementation systems were considered independent variables. Bond strength (24 hours or 6 months), failure mode analysis, cementation system thickness, adhesive interface evaluation, and dentinal penetrability were considered dependent variables.

The specimens were randomly allocated into six groups (n=20), according to the cementation system and post customization.

AS-RA-NC (AS: Adper Scotchbond Multi-Purpose; RA: RelyX ARC; NC: non-customized post)—Dentin was etched with 37% phosphoric acid (Condac 37, FGM, Joinville, SC, Brazil) for 15 seconds, rinsed with distilled water for 30 seconds, and dried using absorbent paper points. Then, both primer and adhesive (Adper Scotchbond Multi-Purpose Plus, 3M Oral Care, St. Paul, MN, USA) were applied throughout the dentin length using a rotary brush (MK Life, Porto Alegre, RS, Brazil) with continuous rotary motion at 500 rpm. Only the adhesive was applied in the fiber post. After that, the adhesive system was light cured for 10 seconds using an LED device (Valo, Ultradent, South Jordan, UT, USA) positioned 1 mm distance from the post surface, with an irradiance of 1000 mW/cm^2 (Standard mode). Afterwards, RelyX ARC cement (3M Oral Care) was inserted using a precision syringe (Maquira, Maringá, PR, Brazil). The post was placed in the root canal, and the whole set was light cured in each root surface for 40 seconds (buccal, mesial, distal, and occlusal).

R2–NC [RelyX U200 (R2); noncustomized post (NC)—The root dentin was rinsed with 5 mL of distilled water and dried using absorbent paper points. The surface was kept slightly moist. Then, self-adhesive resin cement (RelyX U200) was handled and inserted using a precision syringe (Precision, Maquira, Maringá, PR, Brazil). The post was placed in the root canal, and the whole set was light cured, as previously described.

SU–RU–NC [Scotchbond Universal (SU); RelyX Ultimate (RU); noncustomized post (NC)]—The root dentin was rinsed with 5 mL of distilled water and dried using absorbent paper points. Two layers of the Universal adhesive system (Scotchbond Universal; 3M Oral Care) were applied on the post space and light cured for 10 seconds, as previously described. Excesses were removed using absorbent paper points, and the dentin was dried by an air spray. Further, the universal adhesive system was applied on the fiber post and light cured for 10 seconds. Afterwards, RelyX Ultimate cement (3M Oral Care) was inserted using a precision syringe. The post was placed in the root canal, and the whole set was light cured using an LED device for 40 seconds, positioned 1-mm distance from the post surface, as previously described.

AS–RA–C, R2–C, and SU–RU–C [customized post (C)]—The cementation was performed according to the manufacturer's recommendations. However, the fiber posts were previously customized in these

protocols. The posts were etched with 37% phosphoric acid (Condac 37, FGM) for 15 seconds, rinsed for the same time, and dried by air spray. Two layers of silane (Prosil, FGM) were applied throughout the post length and dried by air spray. Then, a two-step etch-and-rinse adhesive system (Adper Single Bond 2; 3M Oral Care) was applied on the post length and light cured for 10 seconds.

The post space was lubricated using a water-based glycerin gel (KY Gel; Reckitt Benckiser, Slough, UK). Then, a single increment of resin composite (Z350 XT AT; 3M Oral Care) was applied around the post and inserted into the root canal. Then, the whole set was light cured for 10 seconds. The customized post was removed from the root canal and light cured for 40 seconds on each surface. The post space was irrigated with 10 mL of distilled water to remove the water-based gel and dried using absorbent paper points. Push-out analyses were performed in a period of 24 hours (n=60 specimens) and in a period of 6 months (n=60 specimens) after fiber post cementation.

Table 1 shows the composition of resin cements and adhesive systems used in the cementation protocols.

Rhodamine B (LabSynth, São Paulo, SP, Brazil) was added to the primer of the adhesive system (Adper Scotchbond Multipurpose) and to the universal adhesive system (Scotchbond Universal), in a ratio of 16 μL to 0.4 mL. Rhodamine B was also added to RelyX U200 cement, in a ratio of 0.01% (by mass) in accordance to Bim Júnior and others.[27] Only specimens evaluated at 6 months were subjected to CLSM. The roots were kept in distilled water for 6 months at 37°C ± 1°C. The water was changed every 2 days. After 6 months, push-out testing and dentinal penetrability evaluation were performed.

Bond Strength Evaluation

After 24 hours and 6 months, the roots were vertically centralized inside a PVC matrix (21.3-mm diameter x 20.0-mm length) and checked using a parallelometer (BioArt B2, São Carlos, SP, Brazil). The matrices were filled with polyester resin (Maxi Rubber, Diadema, SP, Brazil), leaving 1.0 mm of the cervical root outside the resin. The whole set was left undisturbed for 24 hours. The specimens were removed from the matrices and then sectioned perpendicular to their long axis using a diamond disk (250 rpm) coupled in a hard tissue cutting machine (IsoMet 1000, Buehler Ltd, Lake Bluff, IL, USA) under water cooling.

Three sections were obtained with 2.0 mm ± 0.1 mm thicknesses from the apical-, middle-, and cervical-thirds of the post space. The cervical, middle, and apical sections were obtained, respectively, from 1.0 mm, 5.0 mm, and 8.0 mm apical to the root cervical face. Section irregularities were removed using 1200-grit silicon carbide sandpaper (Norton, São Paulo, SP,

Table 1: *Composition of Resin Cements and Adhesive Systems used in the Cementation Protocols*

Materials	Manufacturer	Composition
Adper Scotchbond Multipurpose	3M Oral Care	Primer: water, HEMA, copolymer of acrylic and itaconic acid
RelyX ARC	3M Oral Care	Bis-GMA, TEGDMA, silanized zirconia/silica filler 68% functionalized dimethacrylate polymer, triphenyl antimony
RelyX U200	3M Oral Care	Base: glass powder treated silane, 2-propenoic acid, 2-metil 1,1'-[1-(hydroxymetil)-1,2- ethanodlyl] ester, TEGDMA, sodium persulfate and t-butyl per-3,5,5–trimethyl-hexanoate. Catalyst silane-treated glass powder, substituted dimethacrylate, silanated silica, sodium p-toluene sulfonate, 1-benzyl-5-phenyl-baric acid, calcium salts, 1,12-dodecane dimethacrylate, calcium hydroxide and titanium dioxide
Scotchbond Universal	3M Oral Care	MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane
RelyX Ultimate	3M Oral Care	Methacrylate monomers, radiopaque silanated fillers, initiator components, stabilizers and rheological additives, radiopaque alkaline fillers, pigments, fluorescence dye, dark polymerize activator for SU

Abbreviation: Bis-GMA, bisphenol-glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate, MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethylene glycol dimethacrylate.

Brazil) under water cooling. The sections were then ultrasonically cleansed for 5 minutes.

The apical-, middle-, and cervical-thirds were demarcated, and the specimens were submitted to a push-out test using an electromechanical testing machine (EMIC, São José dos Pinhais, PR, Brazil), at 0.5 mm/min speed with 5 kN load cell, until the complete displacement of the fiber post and/or cementation system. Punch diameters of 1.2 mm, 0.9 mm, and 0.5 mm for the cervical-, middle-, and apical-thirds of the post space, respectively, were used.

The force (F) required for the displacement of the specimens was obtained in N (newtons) and transformed into bond strength (MPa) by the formula: MPa=F/AD; whereas AD was the adhesion area to dentin and calculated by the formula: $AD=\varpi \cdot (R + r) \cdot g$, where R = cervical root canal radius (mm); r = apical root canal radius (mm); g = relative height of the inverted cone (mm). Cervical and apical root canal diameters were obtained with a stereo microscope at 20× magnification (Leica Microsystems, Wetzlar, Germany). The value of g was obtained using the formula:

$$g = (R - r)^2 + (2.0)^2.$$

Failure Mode Analysis

Afterwards, the cervical surface of each slice, for the 24-hour and 6-month periods, was polished with alumina (0.3 μm and 0.05 μm granulation, Arotec, Cotia, SP, Brazil) and felt disk (FVL, Arotec), driven on a circular polisher (Aropol VV, Arotec) under water cooling. Then, the specimens were rinsed in an ultrasonic tank for 5 minutes.

The perimeter of the adhesive interface was divided into four quadrants, and an image of each quadrant was obtained using a confocal laser microscope (Lext OLS4100, Olympus, Shinjuku, Tokyo, Japan) at ×1024 magnification. The failure mode was classified according to Ramos and others[17] in type 1 (adhesive 1), when it occurred between the post and the cement; type 2 (adhesive 2), between dentin and cement; type 3 (cohesive), within the cement; and type 4 (mixed), when both types of failure were combined. Figure 1 displays the failure mode analyzed with CLSM.

Cementation System Thickness Analysis

In the groups evaluated after 24 hours, the thickness of the cementation system was evaluated before the push-out test (bond strength). The cervical face of each slice was gradually polished with 600-grit and 1200-grit silicon carbide sandpapers (Norton), in a circular polisher (Arotec) under water cooling. The specimens were rinsed in distilled water, and the cervical surface

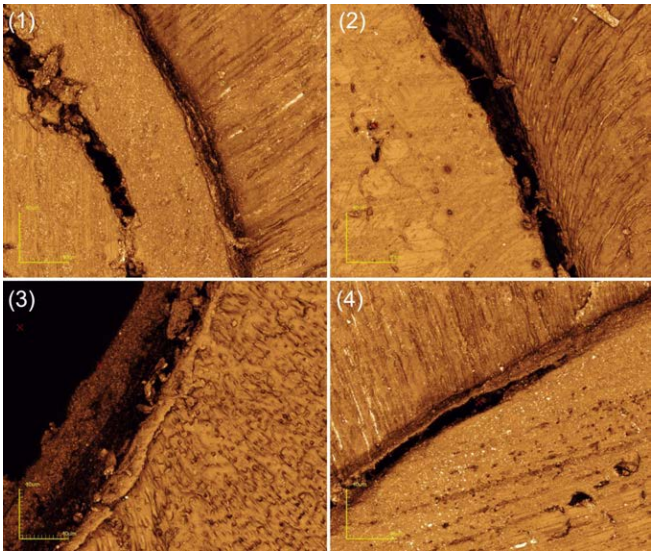


Figure 1. *Representative image of adhesive failure mode: Type 1 (adhesive): When occurring between the post and cement; type 2 (adhesive): Between dentin and cement; type 3 (cohesive): Within cement; and type 4 (mixed): When both types of failure were combined. Scale: 40 μm.*

was polished with alumina (30-μm granulation; Arotec). Then, they were rinsed with distilled water. After drying, each specimen was analyzed with CLSM (Lext OLS4100) using an specific software (Olympus Stream, Olympus) at 1024× magnification. To obtain the images, the perimeter of the root canal was divided into four quadrants. From each quadrant, an image was obtained and ten measurements were performed (in micrometers) on this image using the Image J (National Institutes of Health, Bethesda, Maryland, USA) program. The arithmetic average of the forty measurements was considered as the mean of the thickness of the cementation system in each section.

Adhesion Interface Evaluation

Thirty-six roots received the post preparation and were randomly distributed in the six groups evaluated (n=6), as previously described, to evaluate the characteristic of the adhesion interface between the dentin and the cementation system, in the cervical-, middle-, and apical-thirds of the post space. After 4 hours, sections were made, and the specimens were polished with 1200-grit silicon carbide (Norton) sandpaper under water cooling and rinsed in an ultrasonic tank with distilled water for 3 minutes.

Then, the specimens were immersed in 18% hydrochloric acid (6N), for 30 seconds, rinsed in distilled water for 1 minute, immersed in 2.5% sodium hypochlorite solution (Asfer, São Caetano do Sul, SP, Brazil) for 10 minutes, and rinsed again in distilled

water for 10 minutes. After drying the specimens, the cervical face of each specimen was molded with polyvinyl siloxane (Express XT; 3M Oral Care), and epoxy resin-based replicas were obtained (Buehler).

The resin specimens were individually mounted on metallic stubs and coated in gold-palladium (Bal-Tec, Balzers, Liechtenstein) at 20 mA for 180 seconds. Representative images of the adhesion interface were obtained using SEM (JEOL; Peabody, MA, USA) at 2000× magnification. Figures 2 and 3 show the representative images of the adhesion interface between the cementation system at the post space thirds for noncustomized and customized posts, respectively.

Dentinal Penetrability Evaluation

After 6 months, 60 specimens (10 per group) were submitted to CLSM (LSM5; Zeiss, Jena, Germany) analysis. The images of the root canal diameter were divided into four quadrants. An image of each quadrant of the adhesive interface perimeter was obtained at 10× magnification. 10 measurements of dentinal penetrability were considered in each quadrant—a total of 40 measurements. The arithmetic average of the quadrant measurements was considered as the mean of each specimen. The measurements of the cement penetration into the dentinal tubules were performed in each quadrant using the Image J program.

Statistical Analysis

Bond strength (24 hour and 6 month), cementation system thickness, and dentinal penetrability data were submitted to Shapiro–Wilk testing. Then, the data were subjected to two-way ANOVA followed by Tukey post-hoc tests (α=0.05). The failure mode data were classified according to their incidence in the post space third.

RESULTS

Bond Strength Evaluation

24 Hours—In all thirds, the protocols with customized fiber posts (AS-RA-C, R2-C, and SU-RU-C) showed the highest bond strength (p<0.05) but similar to each other (p>0.05). In the cervical- and middle-thirds, R2–NC presented the lower bond strength (p<0.05), but AS–RA–NC and SU–RU–NC presented similar values (p>0.05). In the apical-third, the protocols without a customized fiber post presented similar bond strength between them (p>0.05).

6 Months—The protocols with customized fiber posts (AS–RA–C, R2–C, and SU–RU–C) presented the highest bond strength, regardless of the dentin-third and the luting system (p<0.05), and they were similar among them (p>0.05).

The analysis by thirds showed that R2–NC and SU–RU–NC protocols presented the lowest bond strength in the middle- and apical-thirds (p<0.05). Moreover, in the cervical-third, bond strength was similar to the AS–RA–NC protocol (p>0.05).

The customized variable was significant in all groups, and the variable thirds were only significant in groups R2–NC and SU–RU–NC (p<0.05).

Table 2 shows the mean and standard deviation of the bond strength values of the cementation system to dentin according to the customization protocol, post space thirds, and periods of evaluation.

24 Hours × 6 Months—Push-out bond strength was compared between the periods of 24 hours and 6 months exclusively within each group. In the cervical-third, all groups presented a reduction in bond strength after 6 months. In the middle-third, a reduction in bond strength was only observed after 6 months in the noncustomized fiber post groups (p<0.05). In the apical-third, no reduction was observed, regardless of the group (p>0.05).

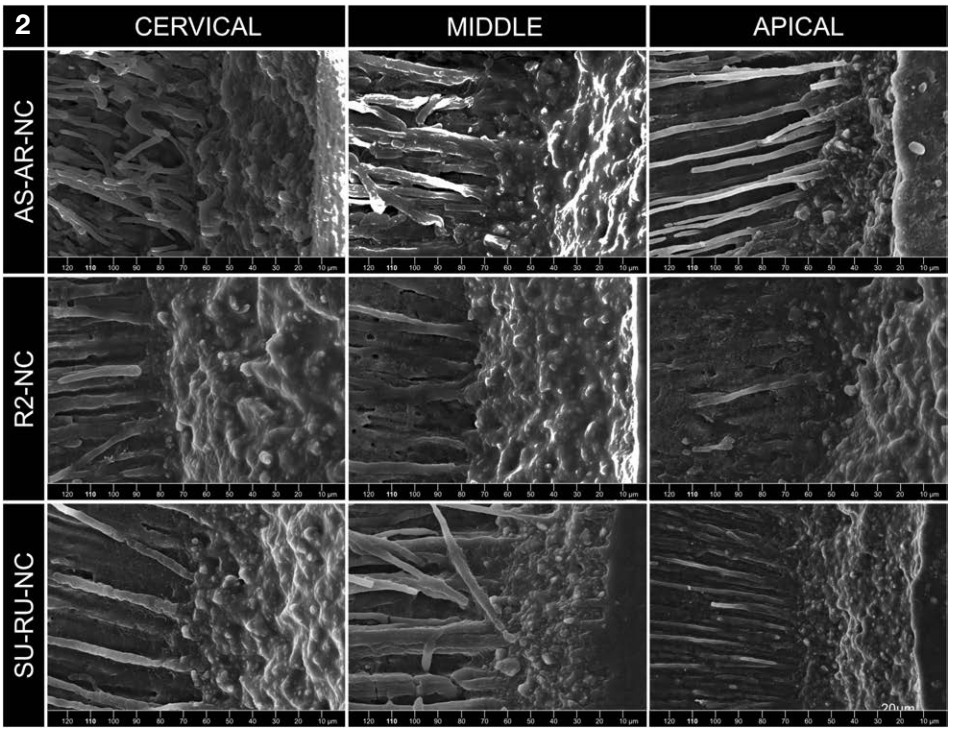
Failure Mode Analysis

24 Hours—Type 4 (mixed) failure mode was the most frequent in all groups for cervical- and apical-thirds. However, for the middle-third, type 3 failure mode was more frequent in noncustomized post groups, while type 4 was more frequent in customized post groups protocols. Figure 4 shows the failure mode in all protocols in the 24-hour period.

6 Months—Type 4 (mixed) failure mode was higher in the noncustomized posts protocols, regardless of the post space third and luting system. On the other hand, the customized posts protocols showed that type 4 failure mode was more frequent in the cervical-third, whereas type 1 failure (post/cement) presented the highest incidence in the middle- and apical-thirds. Figure 5 shows the failure mode in all protocols in the 6-month period.

Cementation System Thickness Analysis

In all thirds, customized post protocols presented the lowest cementation system thickness, regardless of the cementation system or post space third (p<0.05). However, these protocols presented similar measures between them (p>0.05). On the other hand, there were no differences in cementation system measures between noncustomized (NC) post protocols (p>0.05). Table 3 shows the mean and standard deviation of the cementation system thickness according to customization protocol and post space thirds.



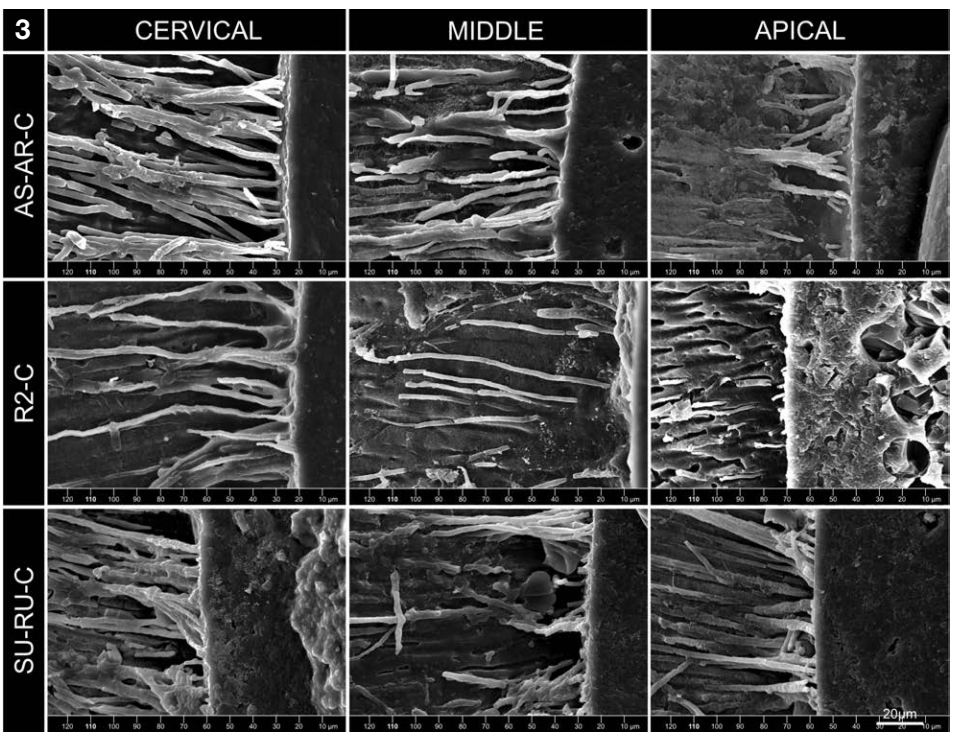


Figure 2. *Representative image of the adhesion interface among cementation system with noncustomized fiber post and radicular dentin, in "space post thirds." The ruler is only for reference. Magnification: 2000×; scale: 200 µm (24 h). AS, Adper Scotchbond Multi-Purpose; C, customized post; NC, noncustomized post; R2, RelyX U200; RA, RelyX ARC; RU, RelyX Ultimate; SU, Scotchbond Universal.*

Figure 3. *Representative image of the adhesion interface among cementation system with customized fiber post and radicular dentin, in "space post thirds." The ruler is only for reference. Magnification: 2000x; Scale: 200 µm (24 h). AS, Adper Scotchbond Multi-Purpose; C, customized post; NC, noncustomized post; R2, RelyX U200; RA, RelyX ARC; RU, RelyX Ultimate; SU, Scotchbond Universal.*

Table 2: *Mean and Standard Deviation (MPa) of the Bond Strength Values of Cementation Protocols, According to Time Evaluation, the "Space Post-third" and Resin Cements*[a]

		AS–RA–NC	R2–NC	SU–RU–NC	AS–RA–C	R2–C	SU–RU–C
24 hours	Cervical	7.51 ± 0.34 bA	6.83 ± 0.54 bA	7.57 ± 0.90 bA	8.62 ± 0.35 aA	8.61 ± 0.36 aA	8.66 ± 0.43 aA
	Middle	6.76 ± 0.31 bA	5.12 ± 0.55 bA	6.83 ± 0.74 bA	7.79 ± 0.49 aA	7.63 ± 0.45 aA	7.75 ± 0.45 aA
	Apical	5.71 ± 0.57 bA	5.63 ± 0.33 bA	5.78 ± 0.45 bA	7.52 ± 0.47 aA	7.45 ± 0.51 aA	7.57 ± 0.63 aA
6 months	Cervical	5.58 ± 0.39 bB	5.07 ± 0.73 bB	5.27 ± 0.54 bB	7.66 ± 0.36 bB	7.32 ± 0.58 bB	7.39 ± 0.40 bB
	Middle	5.52 ± 0.57 bB	4.66 ± 0.64 cB	4.73 ± 0.35 cB	7.46 ± 0.67 aA	7.29 ± 0.60 aA	7.25 ± 0.29 aA
	Apical	5.50 ± 0.36 bA	4.49 ± 0.72 cA	4.43 ± 0.93 cA	7.21 ± 0.61 aA	7.08 ± 0.47 aA	7.13 ± 0.66 aA

Abbreviation: AS, Adper Scotchbond Multi-Purpose; C, customized post; NC, noncustomized post; R2, RelyX U200; RA, RelyX ARC; RU, RelyX Ultimate; SU, Scotchbond Universal.

[a]*Different lowercase letters on the same line indicate significant differences in the bond strength (*p*<0.05). Different uppercase letters in the same column and third indicate significant differences in the bond strength (*p*<0.05)*

Dentinal Penetrability Evaluation

AS–RA protocols presented the greatest dentinal penetrability, regardless of the dentin third and post customization ($p<0.05$). In contrast, the R2–NC protocol (RelyX U200 cement with noncustomized post) showed the lowest dentinal penetrability ($p<0.05$); however, the R2–C protocol with customized posts presented dentinal penetrability similar to Scotchbond Universal/RelyX Ultimate protocols ($p>0.05$).

Table 4 shows the mean and standard deviation of dentinal penetrability values of the cementation system to dentin according to the customization treatment. Figures 6 and 7 display representative images of dentinal penetrability of the cementation systems in the post space thirds, using both noncustomized and customized posts.

DISCUSSION

Customized posts luted with traditional protocols using alternative resin cements presented a positive effect on the bond strength and dentinal penetrability. The lowest cement system thickness was observed when conventional resin cement with etch-and-rinse, universal, and self-adhesive systems were used. Therefore, the null hypotheses were rejected.

Proper sealing of the root canal is essential for successful endodontic treatment. The use of customized anatomical fiber post during the rehabilitation procedure ensures better adaptation to the walls of the root canal, decreases the risks of axial displacement, and, consequently, the risks of adhesive failures and reinfection of the root canal.[7,8]

After 24 hour and 6 month immersion in distilled water, the cementation systems in the customized posts presented similar bond strengths. In addition, customized fiber posts presented the highest push-out values. These results may be explained by two factors: higher frictional retention of the fiber posts and lower thickness of the cement layer.[21,28,29] These data are in accordance to Macedo and others[7] who observed that customization increased fiber post retention due to the improved contact between the cement and adhesive.

The customized fiber post with resin cement presented low shrinkage stress, since the volume and layer of cement were reduced.[29-31] This study showed that the bond strength values were in accordance to D'Arcangelo and others,[32] although, the luting systems presented different bonding strategies, as shown in Table 1.

The bonding between dental materials and root dentin is commonly assessed using push-out tests.[15,33-35] Despite it not showing the real clinical behavior of resin cements, push-out testing is one of the most used methods to evaluate the bonding of dental materials.[2,17,36,37] However, many variables may affect the bond strength results, such as, the material stiffness, specimen position in relation to the load force application, and the diameter of the root canal and punch.[36] The bond strength may also be affected when the material under evaluation undergoes deformation.[37] Nevertheless, it was not relevant in this study, since the whole set (cementation system/fiber post) was rigid and, practically, did not suffer plastic deformation during the push-out test.

Misalignment during the inclusion of the specimens in polyester resin results in deviation of the load application, causing significant variation in bond strength values.[36] The adhesive area is obtained using the following formula:

$$A=\pi \bullet g \bullet (R+r),$$

where g is obtained from the formula $g=(R-r)^2+(h)^2$. If the h value (circular straight cone trunk height) is not constant due to the lack of uniformity in the specimens, the bond strength values will be severely compromised.[34,35] In order to avoid this, the specimens

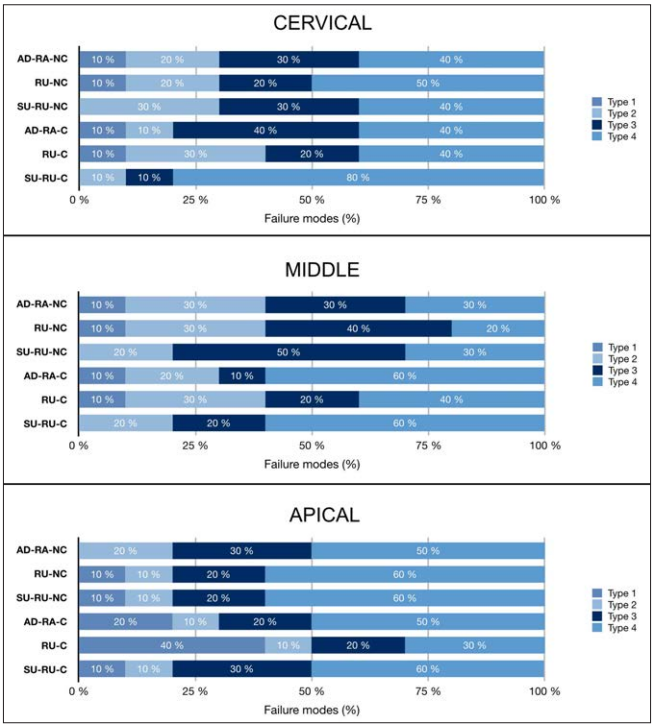


Figure 4. *Incidence of the failure mode according to the cementation protocols and post space thirds (24 hours).*

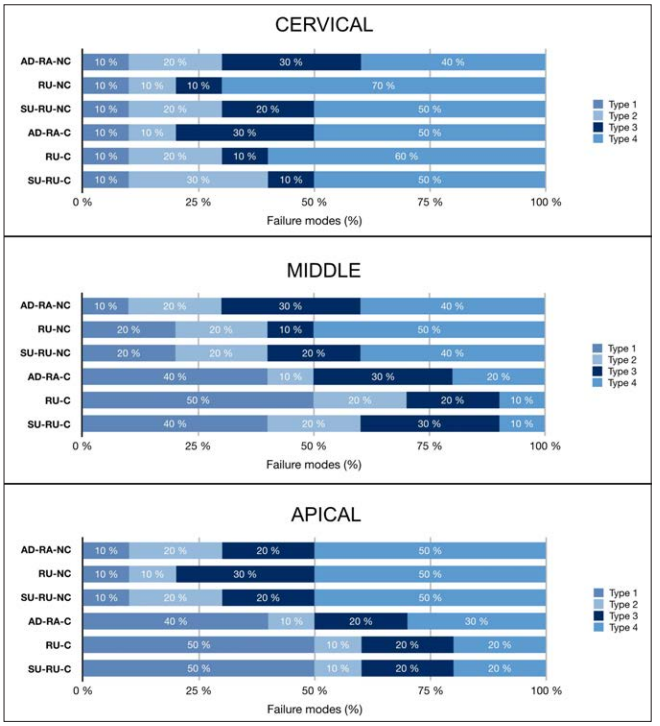


Figure 5. Incidence of the failure mode according to the cementation protocols and post space thirds (6 months).

were included in vinyl resin using a parallelometer. Then, they were transversely sectioned to their root axis with the same thickness and individually checked with a caliper (0.01 mm).

The punch diameter should be between 50% to 83% of the root canal diameter.[36,38] Thus, this study used a punch with compatible diameters for each post space third, in order to avoid any influence on the bond strength values. The diameters of 1.2 mm, 0.9 mm, and 0.5 mm for the cervical-, middle-, and apical-thirds, respectively, were used. Additionally, it avoided friction with the root dentin and plastic deformation of the material.

The bond strength values are also associated with the type of cement, its thickness, and its polymeric degree of conversion.[7,39] Noncustomized posts placed in wide root canals increase the cement thickness and, depending on the cement composition, may affect the its polymerization in deeper regions, reducing the bond strength and the dentinal penetrability.[39]

In all root-thirds, customized fiber posts presented the lowest cement system thickness, but no differences between the cementation protocols were observed due to the adaptation in the root canal.[21,28,29] On the other hand, the cementation system thickness in the groups of noncustomized fiber post were similar to each other, because the post space was subjected to conformation with the DC#2 bur, which has a similar taper to the fiber post used in this study. In the push-out analysis, all noncustomized groups showed lower bond strength values than customized groups, except in the cervical-third for the 6 month evaluation. However, in that

Table 3: *Mean and Standard Deviation (in micrometers) of the Cementation System Thickness According to Customization Protocol and "Space Post-thirds"*[a]

	AS–RA–NC	R2–NC	SU–RU–NC	AS–RA–C	R2–C	SU–RU–C
Cervical	53.53 ± 3.96 b	54.84 ± 4.07 b	53.04 ± 2.37 b	8.65 ± 1.55 a	8.66 ± 1.21 a	8.31 ± 0.92 a
Middle	28.88 ± 1.52 b	29.33 ± 1.39 b	28.43 ± 1.28 b	7.45 ± 0.49 a	7.58 ± 0.45 a	7.41 ± 0.37 a
Apical	14.57 ± 1.01 b	14.62 ± 0.82 b	14.18 ± 0.67 b	4.57 ± 0.68 a	7.28 ± 0.25 a	4.47 ± 0.34 a

Abbreviations: AS, Adper Scotchbond Multi-Purpose; C, customized post (6 minutes); NC, non-customized post; R2, RelyX U200; RA, RelyX ARC; RU, RelyX Ultimate; SU, Scotchbond Universal.

[a]*Different lowercase letters on the same line indicate significant differences in the cement system thickness (p<0.05).*

Table 4: *Mean and Standard Deviation (mm) Values of Dentinal Penetrability According to Space post-third and Type of Cementation System*[a]

	AS–RA–NC	R2–NC	SU–RU–NC	AS–RA–C	R2–C	SU–RU–C
Cervical	383.22 ± 27.08 a	123.27 ± 14.80 c	217.17 ± 20.85 b	377.70 ± 16.72 a	209.45 ± 10.52 b	216.6 ± 14.18 b
Middle	376.97 ± 12.95 a	84.09 ± 13.06 c	208.82 ± 9.22 b	375.89 ± 13.92 a	203.74 ± 11.06 b	207.20 ± 12.85 b
Apical	369.87 ± 11.30 a	82.57 ± 10.98 c	206.74 ± 6.21 b	370.84 ± 9.05 a	199.81 ± 12.77 b	203.88 ± 11.45 b

Abbbreviations: AS, Adper Scotchbond Multi-Purpose; C, customized post (6 minutes); NC, noncustomized post; R2, RelyX U200; SU, Scotchbond Universal; RA, RelyX ARC; RU, RelyX Ultimate.

[a] *Different lowercase letters on the same line indicate significant differences in the dentinal penetrability ($p<0.05$).*

situation, the mixed failure mode was the most frequent and indicates that the rupture probably occurred directly in the resin cement. Therefore, due to this phenomenon, the results were similar regardless of the cementation system thickness.

The dentinal penetrability evaluation using CLSM images can quantify the cementation system diffusion into the dentinal tubules and the collagen matrix.[15,27] This study used a conventional cementation system with etch-and-rinse (Adper Scotchbond Multi-Purpose), Universal (Scotchbond Universal), and self-adhesive systems. Rhodamine pigment was added to the adhesive system using a ratio of 16 μL to 0.4 mL of primer (Adper Scotchbond Multi-Purpose) or Universal adhesive (Scotchbond Universal), and 0.1% (mass/mass) in the self-adhesive cement (RelyX U200) to be analyzed by CLSM.[2,16,17] Although the fluorescent pigments may reduce the monomer conversion and the bond strength of resinous compounds to dentin, the concentrations used in this study presented no effect on the polymerization, the dentinal penetrability, and the bond strength of resin-based materials.[27,29,40]

Adhesive interface analysis using CLSM presents an advantage over scanning electron microscopy (SEM), since CLSM enables the preservation of the specimens and does not require a vacuum during image processing, which avoids technical artifacts, such as cracks and false areas of misadaptation.[28,29] Thus, this study has used surface laser confocal microscopy (Lext OLS4100) to analyze the adhesive failure mode, after identification with the CLSM evaluation.

According to our results, dentinal penetrability occurs according to the dentin substrate and the adhesive system, in addition to the pressure exerted on the

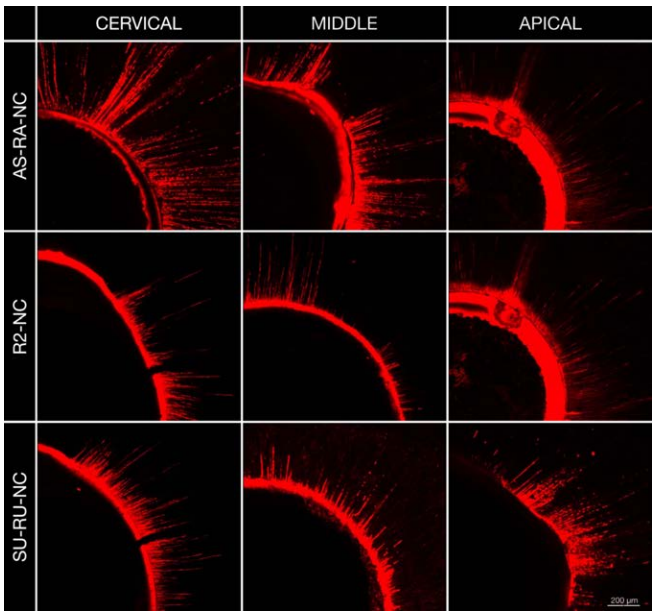


Figure 6. *Representative image of the cementation system penetration in dentin, according to the protocol using non-customized posts and root third. Scale: 100 µm (6 mm). AS, Adper Scotchbond Multi-Purpose; RA, Relyx ARC; R2, Relyx U200; SU, Scotchbond Universal; RU, Relyx Ultimate; NC, non-customized.*

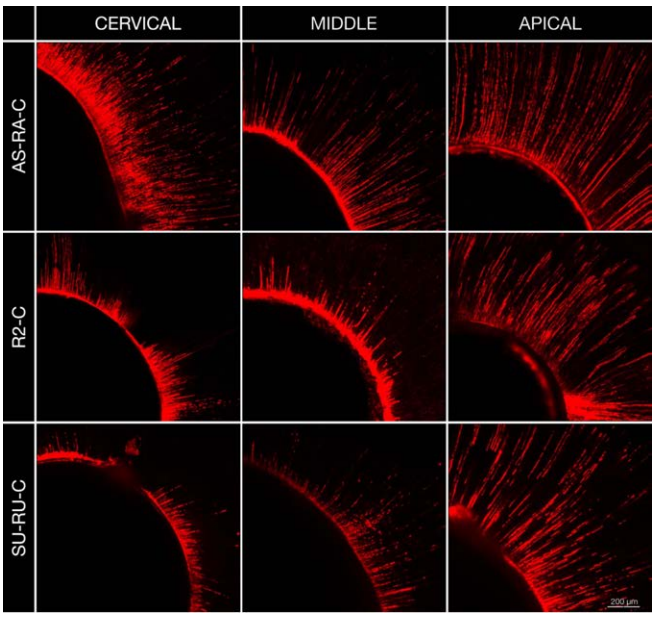


Figure 7. *Representative image of the cementation system penetration into the post space dentin, according to the protocol with customized posts and root third. Scale: 100 µm (6 mm). AS, Adper Scotchbond Multi-Purpose; NC, non-customized; RA, Relyx ARC; R2, Relyx U200; SU, Scotchbond Universal; RU, Relyx Ultimate.*

walls. Although the self-adhesive resin cement system does not have a greater penetrability, it shows relative adhesion mainly by a chemical process.[7,18] A three-step etch-and-rinse adhesive system showed higher dentinal penetrability than a one-step self-etching system (Universal) and a self-adhesive cementation system (RelyX U200), due to its primer, presenting lower surface tension.[13,15]

Conventional cementation systems presented higher dentinal penetrability than self-adhesives, because fluorescent pigment was added to the most fluid substance, such as, into the primer (Adpter Scotchbond Multi-Purpose) or directly in the Universal adhesive (Scotchbond Universal). However, it did not show a direct relation to the post retention in the root canal, according to Lorenzetti and others.[2]

Customization provides a smaller volume of cement and, due to the better adaptation to the fiber post space, possibly also provided greater pressure during the insertion of the post, which may influence the similarity of results regarding dentinal penetrability.[7] On the other hand, the polymerization of self-adhesive resin cement and consequently its bond strength to dentin may have been compromised due to the interference of residual acid monomers on the tertiary amines in the dentin area of the prosthetic space triggered by incomplete photoactivation.[39]

Therefore, resin customization appears to be a viable alternative to minimize the risks of axial displacement of the fiber posts and to improve the clinical longevity of restorative procedures in endodontically treated teeth. However, further studies should be conducted in order to improve and complement this study, such as, which type of resin composite should be used in the customization procedure and the photoactivation timing of cementation systems.

CONCLUSIONS

Customized fiber posts presented better bond strength of the cement, enhanced dentinal penetrability, and a lower cementation system thickness than noncustomized posts, regardless of the cementation protocol tested.

Acknowledgments

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Regulatory Statement

This study was approved by the Ethical Committee in Animal Use from the Araraquara School of Dentistry (23/2019).

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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Evaluation of Dentin Tubule Plugging Efficiencies and Effects on Dentin Surface Roughness of Dentin Desensitizing Agents, the Er,Cr:YSGG Laser, and Their Combination After Erosion-abrasion Cycles: An *In Vitro* Study

E Okur • GB Eyüboğlu

Clinical Relevance

Combined laser–DDA treatments could be more effective than DDA treatments alone for dentin hypersensitivity (DH) treatment, particularly in challenging oral conditions, such as erosion and abrasion. These applications may help obtain longer-lasting and more satisfying results in the treatment of DH.

SUMMARY

Objectives: The purposes of this *in vitro* study were to evaluate the tubule plugging efficiencies and effects on the surface roughness of dentin of different dentin desensitizing agents (DDAs; Teethmate Desensitizer, Kuraray; Gluma Desensitizer, Kulzer; Clinpro White Varnish, 3M ESPE; Enamelast, Ultradent) and the Er,Cr:YSGG laser (Biolase, Waterlase), both alone and in combination with DDAs, after application and after an erosion-abrasion cycle.

Methods and Materials: For surface roughness examinations, superficial buccal dentin specimens were divided into 10 groups: the control, Teethmate

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Desensitizer, Gluma Desensitizer, Enamelast, Clipro White Varnish, Er,Cr:YSGG Laser, Teethmate Desensitizer-Laser, Gluma Desensitizer-Laser, and Enamelast-Laser, and Clinpro White Varnish-Laser groups. Profilometric analyses and scanning electron microscopy (SEM) examinations were performed after applications and after a 5-day erosive-abrasive cycle. For the statistical analysis of surface roughness measurements, 2-way analysis of variance (ANOVA), 1-way ANOVA, and Tukey *post hoc* test were used.

Results: Among the treatments, only DDAs alone did not cause increase in surface roughness after application. All of the laser applications increased the surface roughness of dentin, and after the erosion-abrasion cycle, all of the test groups had increased surface roughness. However, SEM images showed that morphological changes were less frequently observed in all of the experimental groups than in the control group. In addition, all of the laser-DDA combinations had stronger tubule occlusion effects than did DDAs alone, even after erosion-abrasion.

Conclusions: All of the test treatments showed protective effects on dentin surfaces against the negative effects of erosion-abrasion. The addition of the laser to DDA applications increased tubular plugging efficiencies of DDAs, and the tubule plugs of the combination treatments were resistant to the erosion-abrasion cycle.

INTRODUCTION

Dentin hypersensitivity (DH) is defined as short-term and sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic, or chemical stimuli that stops after stimulus removal and is not associated with other dental defects or diseases.[1-3] DH has a reported prevalence of between 4% and 74% in the general population.[4] The prevalence varies between 72% and 98% among individuals with periodontal disease.[2] It is most often found in permanent canines and premolars in both dental arches. The cervical facial region of the teeth is the most affected region.[4,5]

DH occurs when dentin tubules are exposed to the oral environment as a result of the loss of enamel and/or root surface.[5] Dentin can become exposed for various reasons. In some developmental tooth anomalies, the enamel tissue, which normally covers the dentin anatomically, cannot contact the cementum at the cervical area, so the dentin tissue is exposed.[6] Gingival recession is another important reason for dentin exposure. Gingival recession and subsequent exposure of the root surface lead to the exposure of the dentinal tubules.[7] Enamel or cementum covering dentin surface can disappear as a result of abrasion, attrition, abfraction, or erosion.[5,6] The consumption of acidic foods and beverages, which are frequently included in today's diets, can also cause enamel loss and exposure of dentin tissue. Gastric acid, with internal causes such as recurrent vomiting, regurgitation, and reflux, can also come into contact with teeth and expose dentin.[8] *In vitro* and clinical studies of DH have shown that acid erosion and tooth brushing can open and widen the dentinal tubules, resulting in the emergence of DH, an increase in its severity, or a decrease in the effectiveness of treatment.[2,9,10]

The most widely accepted theory for DH is the hydrodynamic theory suggested by Brännström and others.[11,12] According to this theory, the fluid inside the dentinal tubules is affected by thermal, physical, or osmotic changes, and these fluid movements stimulate baroreceptors and cause DH.[6] One of the main strategies for treating DH is to occlude the dentinal tubules and thus prevent fluid flow.[13] There are many treatment alternatives for DH, and desensitizing agents with different effect mechanisms have been placed on the dental market.[14] Dentin desensitizing agents (DDAs) can be applied by dentists or by patients at home. Today, the most commonly used DDAs by dentists include dentin tubule occlusive agents and tubule sealant agents.[15] In addition, lasers have been used as an alternative to these agents.[16]

Fluoride varnish applications, which are among the treatments applied by dentists to treat DH, are widely used. Fluoride varnishes contain high fluoride concentrations that can create a mechanical barrier on exposed dentin.[17] Sodium fluoride (NaF, 5%) is used clinically for DH treatment.[18] Topical NaF applications allow for the deposition of calcium fluoride (CaF_2) on the tooth surface, blocking open dentinal tubules and thus reducing dentin permeability.[18-20] Although clinical studies have supported the beneficial results of fluoride, several clinical studies have suggested that fluoride has limited efficacy.[21,22] The slow dissolution of the formed CaF_2 precipitates in saliva, and the small size of these crystals (approximately 0.05 μm) can cause the barrier to become transient.[4,23]

The combination of NaF with chemicals such as tricalcium phosphate (TCP) has been developed since NaF application is not fully effective in occluding the diameter of the dentinal tubules of sensitive teeth and requires repeated applications. Studies have reported that adding TCP to fluoride increases fluoride

retention in both enamel and dentin and facilitates remineralization.[24,25] In addition, according to the manufacturer of a product containing 5% NaF and TCP, the combination causes the release of calcium and fluoride ions when in contact with saliva,[26] and it has been shown to cause the partial occlusion of dentinal tubules and thereby decrease DH in different studies.[26,27]

Calcium phosphate-containing desensitizers have become a popular topic for biological material research in recent years because of their biocompatibility, bioactivity, and crystal structure similar to that of human teeth.[28] Calcium phosphate-containing desensitizers contain tetracalcium phosphate and dicalcium phosphate anhydrous (DCPA), which can spontaneously form hydroxyapatite (HA). This type of desensitizer has been shown to form a calcium phosphate-rich layer on the dentin surface and thereby decrease dentin permeability. It has also been reported to significantly decrease DH by providing remineralization of early enamel lesions.[29,30] Short- and long-term clinical studies have shown that calcium phosphate-containing desensitizers are effective in reducing DH.[31,32]

Hydroxyethyl methacrylate (HEMA) and glutaraldehyde-containing desensitizers block dentin tubules and show rapid and long-term activity. While HEMA physically occludes the dentinal tubules, glutaraldehyde causes coagulation of plasma proteins in the dentinal tubules. Glutaraldehyde primarily reacts with serum albumin in dentinal tubular fluid, causing albumin to precipitate. It then reacts a second time with albumin and results in the polymerization of HEMA. HEMA contributes to the formation of deep resin tags within dentinal tubules owing to its hydrophilic property.[4,33] Scanning electron microscopy (SEM) and confocal laser scanning microscopy studies have shown that a desensitizer containing HEMA and glutaraldehyde blocked dentinal tubules through protein coagulation.[32,34] Clinical and *in vitro* studies have shown that its success rate in reducing DH varies between 5% and 27%.[28,32,34]

With the development of laser technology, a new treatment option has emerged for DH. The Er,Cr:YSGG laser is a medium-power laser that can be used in soft and hard tissues without damaging the pulp and surrounding tissues because of the specific properties of its wavelength (2.78 μm). The Er,Cr:YSGG laser can cut enamel and dentin because of its high absorption in water and its strong absorption by hydroxyl radicals in the HA structure.[35,36] This laser causes insoluble salts to accumulate in the dentinal tubules by evaporation of the dentinal tubular fluid. There have been clinical and *in vitro* studies reporting that this accumulation enables the occlusion of dentinal tubules and the reduction of DH.[37-40] Considering the increase in the prevalence of DH in recent years, alternative treatments are needed that provide long-term efficacy. For this reason, the combined application of DDAs with lasers has been explored for the treatment of DH.[41]

Studies have shown the effectiveness of the combined use of DDAs and lasers in the treatment of DH.[28,39] However, it has been reported that laser applications can cause cracks and irregularities on dentin surfaces.[28] Moreover, factors such as erosion and abrasion can change the surface properties of tooth tissues, which can increase surface roughness.[42] A significant increase in surface roughness causes plaque retention and an increase in bacterial adhesion on dental tissues, creating a surface prone to caries formation.[42,43] Caries formation is one of the most important processes affecting the survival rate of teeth in the mouth.

A limited number of studies have investigated the effects of DDAs, laser application, and the combined application of DDAs and lasers on surface roughness after erosion-abrasion cycles.[26,39] Moreover, to the authors' knowledge, no comprehensive study comparing all of these agents has been conducted.

Therefore, the aim of this study was to investigate the effects of DDA applications with different contents (calcium phosphate; HEMA and glutaraldehyde; 5% NaF and TCP 5%) and Er,Cr:YSGG laser, which can cause insoluble salts to accumulate in the dentinal tubules via the evaporation of the dentinal tubular fluid and thereby enable dentinal tubule occlusion and DH reduction and DDA–laser combinations on dentin surface roughness and tubule plugging efficiency.

Null hypotheses of this study were as follows:

1. DDA applications, laser application, and combination applications to the dentin surface do not have significant effects on surface roughness.
2. DDA applications, laser application, and combination applications do not have significant effects on surface roughness after an erosion-abrasion cycle.

METHODS AND MATERIALS

In this *in vitro* study, calcium phosphate-containing, HEMA- and glutaraldehyde-containing, 5% NaF- and TCP-containing, and calcium- and fluoride-releasing DDAs and Er,Cr:YSGG laser, which enables dentinal tubule occlusion and DH reduction, were applied separately or in combination to dentin specimens.

Power Analysis

Power analysis revealed that the minimum sample size required to detect a significant difference was 9 per

group (90 in total) assuming a type I error (alpha) of 0.05, a power (1-beta) of 0.8, and an effect size of 2 for after the erosion-abrasion cycle. We used 12 specimens for each group.

Specimen Preparation

A total of 140 intact, permanent third molar teeth without caries or cracks extracted for orthodontic or oral reasons were used for this study. Before the extractions, the patients were informed that their teeth would be used for research purposes, and a consent form was read and signed by each patient. After extraction, soft tissue residues and bone particles on the teeth were removed with a periodontal curette. The teeth were maintained in 0.1% thymol solution before the experiments. The buccal surfaces of the teeth were cut vertically in the mesiodistal direction under water cooling with the aid of a low-speed precision cutting device (Micra Cut 125, Metkon, Bursa,Turkey) and 0.3-mm-thick diamond discs (Diamond cut-off wheel B 102, ATM GMBH, Mammelzen, Germany). The enamel tissue was removed, and the superficial dentin tissue was exposed. Then the teeth were cut horizontally from the apex of the enamel-cementum junction to obtain dentin specimens, which were embedded in autopolymerizing acrylic resin (Imicryl, SC, Konya, Türkiye) for use in the experiments. Test specimens were sanded using 600, 800, 1200, 1500, and 2000 grit silicon carbide abrasive papers in a polishing machine (Beta Grinder Polisher, Buehler, IL, USA) with a 200-RPM rotation speed to form a standard smear layer and obtain a smooth surface.

Application of DDAs, Laser, and DDA–Laser Combinations

Dentin specimens were maintained in 17% ethylenediaminetetraacetic acid (EDTA; Werax, Tunadent, Izmir, Turkey) solution for 5 minutes to open the dentinal tubules and remove the smear layer. The specimens were washed under running water to remove residue and sonicated in distilled water for 5 minutes with an ultrasonic cleaner.

A total of 140 specimens were divided into 10 test groups (n=14) (control, TMD [Teethmate Desensitizer, Kuraray Noritake Dental Inc, Okayama, Japan], GD [Gluma Desensitizer, Heraeus Kulzer, GmbH & Co, Hanau, Germany], EN [Enamelast, Ultradent, South Jordan, UT, USA], CWV [Clinpro White Varnish, 3M ESPE, St Paul, MN, USA], L [Er,Cr:YSGG laser, San Clemente, CA, USA], TMD-L [Teethmate Desensitizer–Er,Cr:YSGG laser], GD-L [Gluma Desensitizer–Er,Cr:YSGG laser], EN-L [Enamelast–Er,Cr:YSGG laser], and CWV-L [Clinpro White Varnish–Er,Cr:YSGG laser]). Two specimens from each group were used for SEM analysis.

DDAs were applied to the TMD, GD, EN, and CWV groups according to the manufacturers' instructions. Er,Cr:YSGG laser application was applied to group L. Er,Cr:YSGG laser application was performed at 0.25 W, 20 Hz, and 12.5 mJ. Laser irradiation was performed in noncontact mode with a pulse width of 140 μs using a 6-mm MZ6 tip with a 600-μm diameter operated in 0% water and 10% air. A total of 20 seconds of irradiation was applied vertically and horizontally (10 seconds each) from the 1-mm irradiation distance to the dentin surface.

In the TMD-L, GD-L, EN-L, and CWV-L groups, DDAs were first applied to the dentin specimens, then Er,Cr:YSGG laser application was performed. No applications were performed in the control group. Manufacturer instructions are reported in Table 1.

Erosion-Abrasion Cycle

For all of the groups, a modified 5-day erosion-abrasion model proposed by Scaramucci and others was used.[9] A 0.3% citric acid solution (pH≈2.45) was used to simulate erosion in the mouth. The specimens were immersed in citric acid solution at room temperature for 2 minutes four times per day without stirring. After each episode of erosion, the specimens were immersed for 60 minutes in artificial saliva (0.213 g/l $CaCl_2 \cdot 2H_2O$, 0.738 g/l KH_2PO_4, 1.114 g/l KCl, 0.381 g/l NaCl, and 12 g/l Tris buffer; pH adjusted to 7 with KOH), rinsed with distilled water, and gently dried with absorbent paper. The erosion-abrasion cycle procedures are reported in Table 2.

A brushing mechanism was created with a pressure of 2 N to brush the teeth. Tooth brushing was performed twice per day for 15 seconds in the middle of the first and last remineralization periods using electric brushes (Oral-B Professional, Braun, Frankfurt, Germany). Oral-B Sensitive (Oral-B Professional) was used as the brush head. Brushing was performed with a slurry made from Colgate Maximum Anti-caries Protection dentifrice and artificial saliva (1:3 w/w) (Colgate-Palmolive, SP, Brazil) for all of the groups. Total exposure time of the specimens to the dentifrice slurries in each brushing episode was 2 minutes. Only one operator performed the tooth brushing procedures. During the night, specimens were stored in a humid environment at 4°C.

Profilometric Analysis

A contact profilometer device (Marsurf PS10, Mahr, Göttingen, Germany) was used for surface roughness measurements. First, profilometric

Table 1: *Manufacturer Instructions*

DDAs	Manufacturer	Composition	Lot Number	Application Instructions
Teethmate Desensitizer	Kuraray Noritake Dental Inc	Powder: tetracalcium phosphate, dicalcium phosphate anhydrous Liquid: water, preservative	041154	Mix the powder and liquid (15 s) carefully, apply with the applicator, rub for 30 s, and rinse with water.
Gluma Desensitizer	Heraeus Kulzer	35% 2-hydroxyethyl methacrylate, 5% glutaraldehyde	K010516	Apply to clean dentin with a cotton pellet or brush and allow it to dwell for 30-60 s. Air dry and rinse.
Clinpro White Varnish	3M ESPE	Sodium fluoride (5%), tricalcium phosphate, xylitol	NA56453	Mix according to the dosage guide and apply to clean and dry dentin.
Enamelast	Ultradent	Sodium fluoride (5%), xylitol	BHFSD	Lightly dry area to be treated. Using a painting motion, apply a thin smooth layer to as many dry tooth surfaces as possible. Gently flow cool water over the teeth.
Er,Cr:YSGG laser	Biolase		18002402	A total of 20 s of irradiation was performed vertically and horizontally from the 1-mm irradiation distance to the dentin.

Abbreviations: DDA, dentin desensitizing agents; TCP, tricalcium phosphate.

analysis was performed after EDTA treatment for sample standardization of all of the groups. Other measurements were performed after DDA applications and at the end of the erosion-abrasion cycle. The dentin specimens were measured in three different areas, and the averages were calculated. The results are expressed in micrometers. The device sensor scans an area of 1.5 mm.

SEM Evaluation

Twenty dentin specimens were prepared as one sample from each group and one sample from each group subjected to an erosion-abrasion cycle to be examined by SEM (EVO LS 10, Zeiss, Oberkochen, Germany). Dentin specimens were covered with a thin layer of gold film. SEM examinations were performed at 5 kV with magnifications between 2000 × and 7500 ×. The effectiveness of the DDA lasers and combination applications on dentinal tubule occlusion was examined by SEM. Then the examined dentin samples were broken vertically, and the effects of the agents on the interface morphology of the dentinal tubules were examined.

Table 2: *Erosion-Abrasion Cycle Procedures*

Steps	Procedures	Application
1	Erosion Remineralization Tooth brushing Remineralization	Citric acid (2 min) Artificial saliva (30 min) Exposure to artificial saliva and toothpaste slurry for 2 min; 15 s of active brushing Artificial saliva (30 min)
2	Erosion Remineralization	Citric acid (2 min) Artificial saliva (60 min)
3	Step 2 repeated.	
4	Step 1 repeated.	
5	Kept in a humid environment at 4°C overnight.	

Statistical Analysis

SPSS for Windows 17.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Descriptive statistics are reported as the mean and standard deviation. The Shapiro-Wilk test was used to determine whether the data conformed to a normal distribution. After ensuring normality, two-way ANOVA (group x time) was applied for repeat samples. One-way ANOVA and Tukey *post hoc* test were used to analyze differences between groups within each time frame. In addition, the *t*-test was used for paired samples while analyzing time-dependent changes within each group. Differences at the level of $p < 0.05$ were considered statistically significant.

RESULTS

Profilometric Analysis Results

Interactions are reported in Table 3. According to the interaction table, significant differences were found among the groups, and significant differences were found within the groups according to time ($p<0.001$).

Comparisons of Surface Roughness Among Baseline (T0), After Application (T1), and After the Erosion-Abrasion Cycle (T2) Within Groups — The mean and standard deviation values of surface roughness and significant differences within groups for all of the evaluation periods are shown in Table 4. For the control group, because the T0 and T1 surface roughness values were the same, no comparison was performed. There was a significant difference between T1 and T2 measurements ($p<0.001$) in the control group. Surface roughness increased in the control group after the erosion-abrasion cycle (Table 4).

While there was no significant difference between T0 and T1 measurements in the TMD, GD, EN, or CWV group ($p>0.05$), there were significant differences between T1 and T2 measurements ($p<0.001$), with surface roughness increasing after the erosion-abrasion cycle in these groups (Table 4).

There was a significant difference between T0 and T1 measurements in each of the L, TMD-L, GD-L, CWV-L, and EN-L groups ($p<0.05$; EN-L, $p<0.001$). Surface roughness values increased in all of the laser-applied groups. In addition, the differences between T1 and T2 measurements were significant in these groups ($p<0.001$). Surface roughness increased after the erosion-abrasion cycle.

Comparisons of the Surface Roughness Among Groups After Application (T1) and After the Erosion-Abrasion Cycle (T2) — There were no statistically significant differences in baseline value among the groups ($p>0.05$). Statistical comparisons of T1 and T2 values among the groups are shown in Table 5.

After application, there was no significant difference in surface roughness among the TMD, DDA-only, and L groups ($p>0.05$). However, compared with the TMD group, the TMD-L group showed a significant increase in surface roughness ($p<0.05$). After the erosion-abrasion cycle, there was no significant difference between the control and DDA-only groups ($p>0.05$). The surface roughness values of the L and TMD-L groups were significantly increased compared to that of the TMD group after the cycle ($p<0.001$).

After application, there was no significant difference in surface roughness among the GD, DDA-only, and L groups ($p>0.05$). Group GD-L showed a significant increase in surface roughness compared with group GD after application ($p<0.05$). After the erosion-abrasion cycle, surface roughness of L and GD-L groups was significantly increased compared to that of the GD group ($p<0.001$).

There was no significant difference in surface roughness after application between the EN group and the DDA-only groups ($p>0.05$). The increases in surface roughness in the L ($p<0.05$) and EN-L ($p<0.001$) groups relative to the values in the EN group were significant. After the erosion-abrasion cycle, the surface roughness of the L and EN-L groups significantly differed from that of the EN group ($p<0.001$).

Table 3: *Two-way ANOVA Within-Groups and Between-Groups Interaction Table*

Source	Type III Sum of Squares	*df*	Mean Square	*F*	Significance
Intercept	25.263	1	25.263	9209.027	$p<0.001$
Group	1.470	9	0.163	59.553	$p<0.001$
Error (Group)	0.302	110	0.003		
Time	6.325	1.344	4.706	2572.677	$p<0.001$
Time x Group	1.590	12.096	0.131	71.850	$p<0.001$
Error	0.270	147.835	0.002		

Abbreviation: ANOVA, analysis of variance.

Table 4: *Comparisons of the Surface Roughness of Different DDAs, Laser, and Combination Applications Within Groups*[a]

Group[b]	Baseline (T0)	After Application (T1)	After Erosion-Abrasion (T2)	Within-Group Evaluation
Control	0.174 (0.032) A	0.174 (0.032) A	0.360 (0.021) B	T1/T2 $p<0.001$
TMD	0.148 (0.031) A	0.154 (0.035) A	0.290 (0.031) B	T1/T2 $p<0.001$
GD	0.147 (0.032) A	0.152 (0.030) A	0.309 (0.028) B	T1/T2 $p<0.001$
EN	0.141 (0.038) A	0.146 (0.036) A	0.300 (0.026) B	T1/T2 $p<0.001$
CWV	0.145 (0.028) A	0.148 (0.038) A	0.315 (0.033) B	T1/T2 $p<0.001$
L	0.179 (0.022) A	0.197 (0.033) B	0.705 (0.068) C	T0/T1 $p=0.013$ $p<0.05$ T1/T2 $p<0.001$
TMD-L	0.180 (0.038) A	0.209 (0.042) B	0.604 (0.078) C	T0/T1 $p=0.016$ $p<0.05$ T1/T2 $p<0.001$
GD-L	0.176 (0.027) A	0.201 (0.028) B	0.681 (0.076) C	T0/T1 $p=0.034$ $p<0.05$ T1/T2 $p<0.001$
EN-L	0.158 (0.031) A	0.221 (0.051) B	0.541 (0.078) C	T0/T1 $p<0.001$ T1/T2 $p<0.001$
CWV-L	0.176 (0.031) A	0.201 (0.035) B	0.417 (0.045) C	T0/T1 $p=0.022$ $p<0.05$ T1/T2 $p<0.001$

Abbreviations: CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; TMD, Teethmate Desensitizer.

[a]*Values with different uppercase letters within a row indicate statistically significant differences at different time intervals (among baseline, after application, and after erosion-abrasion cycle) within a group, as determined by Bonferroni test. The significance of mean differences was evaluated at the 0.05 level.*

[b]*DDA-only applications (TMD, GD, EN, and CWV) did not cause an increase in surface roughness after application (T0-T1;* $p>0.05$*). However, the L and combined DDA-laser applications (TMD-L, GD-L, CWV-L, and EN-L) caused increases in surface roughness (*$p<0.05$*; EN-L,* $p<0.001$*). After the erosion-abrasion cycle, surface roughness was increased in all of the test groups, and there were significant differences between T1 and T2 measurements in all groups (*$p<0.001$*).*

After application, the surface roughness values did not significantly differ between the CWV group and the DDA-only groups ($p>0.05$). Surface roughness was significantly increased in the CWV group compared with that in the L and CWV-L groups ($p<0.05$). After the erosion-abrasion cycle, the surface roughness of the L and CWV-L groups was significantly increased compared to that of the CWV ($p<0.001$).

The L group showed a significant increase in surface roughness compared with that of the EN and CWV groups after application ($p<0.05$). In addition, after application, surface roughness did not significantly differ among any of the combination groups ($p>0.05$).

After the erosion-abrasion cycle, group L showed a significant increase in surface roughness compared to all of the other groups except the GD-L group ($p<0.001$). Among the combined-application groups, the GD-L group had the highest surface roughness, and there were significant differences between the GD-L group and the CWV-L ($p<0.001$), EN-L ($p<0.001$) ,and TMD-L ($p<0.05$) groups. In addition, among the combined-application groups (CWV-L, TMD-L, GD-L, and EN-L), the CWV-L group had the lowest surface roughness ($p<0.001$).

SEM Results

In the SEM images, in contrast to the control group and the other DDA-only groups, the TMD group generally showed widespread tubular occlusion on the buccal surfaces. In the interface examinations, the plugs were observed to extend into the tubules. In the

Table 5: *Comparisons of the Surface Roughness of Different DDAs, Laser, and Combination Applications Among the Groups*[a]

Group	After Application (T1)[b]	After Erosion-Abrasion (T2)[c]
Control	0.174 (0.032)	0.360 (0.021) With L ($p<0.001$) With TMD-L ($p<0.001$) With GD-L ($p<0.001$) With EN-L ($p<0.001$)
L	0.197 (0.033) With EN ($p=0.03$; $p<0.05$) With CWV ($p=0.04$; $p<0.05$)	0.705 (0.068) With TMD ($p<0.001$) With GD ($p<0.001$) With EN ($p<0.001$) With CWV ($p<0.001$) With TMD-L ($p<0.001$) With EN-L ($p<0.001$) With CWV-L ($p<0.001$)
TMD	0.154 (0.035) With TMD-L ($p=0.13$; $p<0.05$)	0.290 (0.031) With TMD-L ($p<0.001$)
TMD-L	0.209 (0.042)	0.604 (0.078) With GD-L ($p=0.02$; $p<0.05$) With CWV-L ($p<0.001$)
GD	0.152 (0.030) With GD-L ($p=0.44$; $p<0.05$)	0.309 (0.028) With GD-L ($p<0.001$)
GD-L	0.201 (0.028)	0.681 (0.076) With EN-L ($p<0.001$) With CWV-L ($p<0.001$)
EN	0.146 (0.036) With EN-L ($p<0.001$)	0.300 (0.026) With EN-L ($p<0.001$)
EN-L	0.221 (0.051)	0.541(0.078) With GD-L ($p<0.001$) With CWV-L ($p<0.001$)
CWV	0.148 (0.038) With CWV-L ($p=0.02$; $p<0.05$)	0.315 (0.033) With CWV-L ($p<0.001$)
CWV-L	0.201 (0.035)	0.417 (0.045)

Abbreviations: CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; TMD, Teethmate Desensitizer.

[a] *In the columns, significant* p-*values according to Bonferroni test, indicating differences among different groups within the same time period, are indicated.*

[b] *After application (T1), surface roughness did not increase among the DDA-only groups (*$p>0.05$*), but there was a significant difference between each DDA group and the corresponding DDA-laser combination group (TMD-TMD-L, GD-GD-L, CWV-CWV-L,* $p<0.05$*; EN-EN-L,* $p<0.001$*). In addition, there was no significant difference in surface roughness among all of the DDA-laser combination groups after application (*$p>0.05$*).*

[c] *After the erosion-abrasion cycle (T2), there was no significant difference among the DDA-only groups, but there was a significant difference between each DDA group and the corresponding DDA-laser combination group (*$p<0.001$*). In addition, the L group showed higher surface roughness than all other groups except the GD-L group (*$p<0.001$*).*

images obtained after the erosion-abrasion cycle, some tubule plugs had been removed, but most of the tubule plugs remained, and the levels of surface deterioration and tubular enlargement were quite low compared to those of the control group (Figures 1 and 2).

The SEM images of the GD group revealed a small number of plugs on the buccal surfaces, which did not completely block the tubules. In the images, most of the tubules were open. In the interface images, although plugs were observed in the dentinal tubule orifices, no plugs were observed extending into the tubules. Although open dentinal tubules were generally observed after the erosion-abrasion cycle, surface deformations and irregularities were far less common in the GD group than in the control group (Figures 1 and 2).

In the SEM images of the EN and CWV groups, the dentin tubules were generally open on the buccal surfaces, but there were closed or narrowed tubules in some areas. In the interface images, plugs in the tubule orifices were observed; some of the plugs extended into the tubules in the EN group, and the presence of an occluding layer on the surface in the CWV group was observed. The SEM images showed that after the erosion-abrasion cycle, the tubules in the EN and CWV groups were generally open, but the levels of deterioration and tubular enlargement on the surfaces were far lower than those in the control group (Figures 1 and 2).

In the laser-treated groups, the SEM images showed that the dentinal tubules were generally narrowed, with some occluded and open dentinal tubules also present. In addition, local short cracks and irregularities were observed on the dentin surface. On the interface images, there were plugs in the dentinal tubules and local depression areas on the surfaces. After the erosion-abrasion cycle, there were still plugged dentinal tubules, and local short cracks and irregularities were still observed on the dentin surface, but they were not more abundant than they were after application, and the extent of degradation and abundance of irregularities on the surface was lower than those in the control group (Figures 1 and 2).

In the SEM images of the TMD-L–treated groups, most of the dentinal tubules were closed and narrowed; rarely, open dentinal tubules were also present. In the interface images, some of the tubule orifices were obstructed, and the plugs progressed toward the inner surface of some dentinal tubules. After the erosion-abrasion cycle, closed or narrowed dentinal tubules were still predominant in the SEM images, but open dentinal tubules were also observed. Surface irregularities were far fewer, and degradation was much lower than those in the control group (Figures 2 and 3).

In the GD-L-treated group, the SEM images revealed closed and narrowed dentinal tubules and partially open dentinal tubules. In the interface images, tubule plugs were apparent in the tubule orifices. The SEM images obtained after the erosion-abrasion cycle showed that closed dentinal tubules remained. Surface irregularities and degradation were far less widespread in the GD-L group than in the control group (Figures 2 and 3).

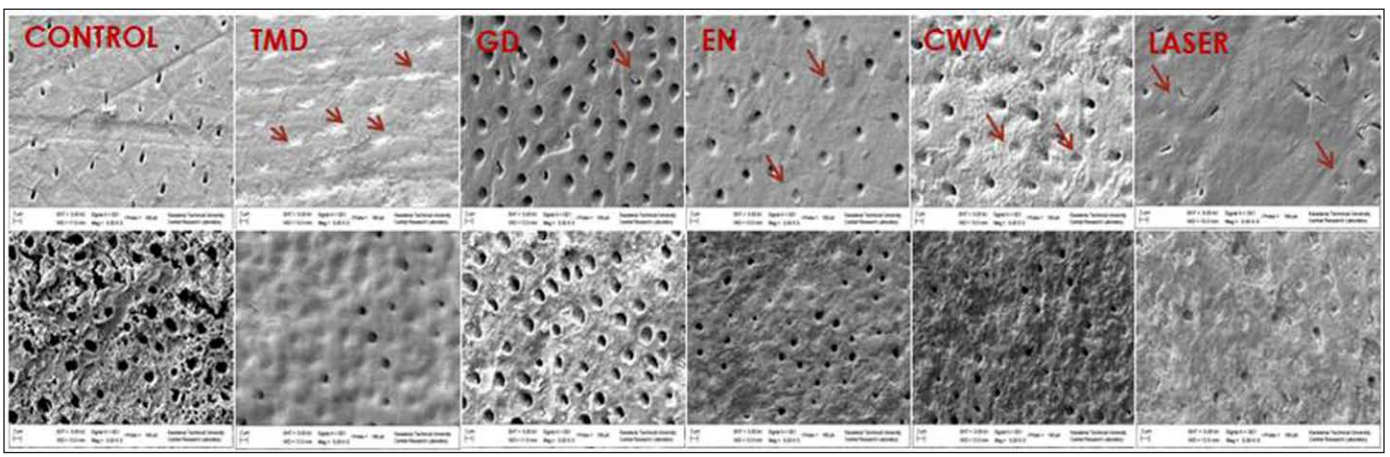

Figure 1. *SEM images of the DDA-only groups and laser-applied groups (the upper images were after the application, and the lower images were after the erosion-abrasion cycle of the same group). According to the after application (upper) images, tubules were occluded in the TMD group. Although there were locally tubular plugs in the GD group, no completely occluded tubules were observed. Although partially occluded tubules were present in the EN and CWV groups, the majority of them were open. There were mostly narrowed, partially occluded tubules, in addition to local cracks and irregularities in the laser group. After the erosion-abrasion cycle (lower images), irregularities on the surface were less frequently observed than in the control group. Arrows indicate occluded dentinal tubules. CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; SEM, Scanning electron microscopy; TMD, Teethmate Desensitizer.*

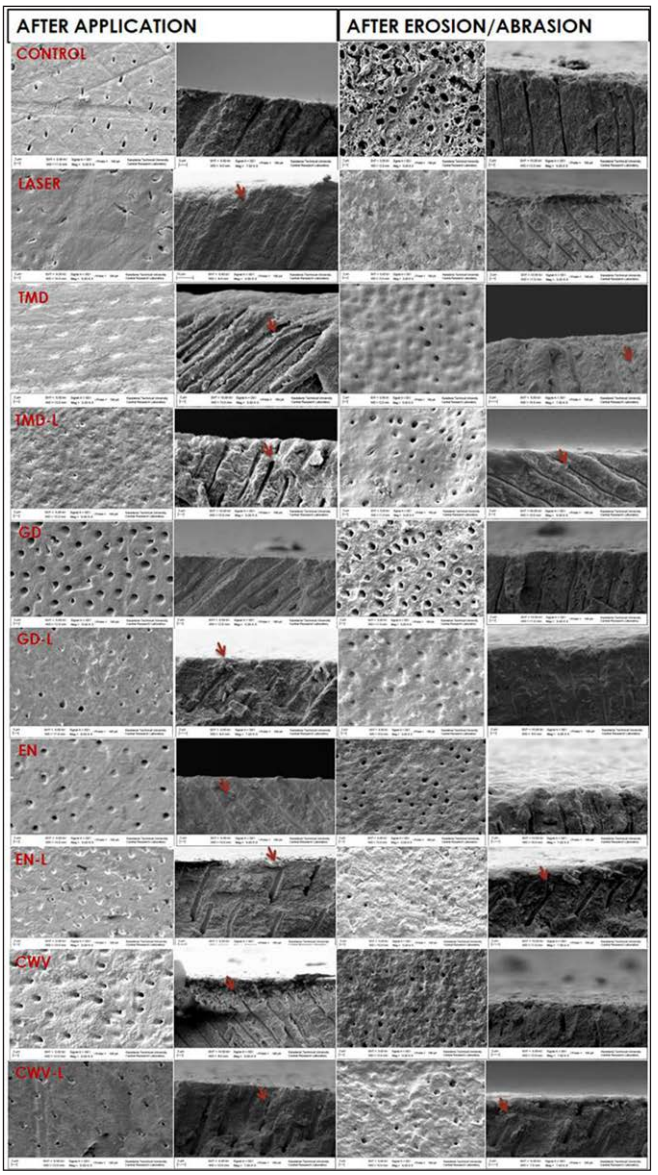

Figure 2. *Comparisons of buccal and interface SEM images after application for all of the groups (only DDA, laser, and combined groups). There were more occluded dentinal tubules in combined applications. Arrows indicate plugs that run inward to the tubules on the interface images. CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; SEM, scanning electron microscopy; TMD, Teethmate Desensitizer.*

In the SEM images of the EN-L and CWV-L groups, the dentinal tubules were generally closed, and plugs in the tubule orifices were observed. In the interface images, tubular plugs were still present on the interface images of both groups. In the SEM images of these groups after the erosion-abrasion cycle, most of the tubule plugs continued to occlude the dentinal tubules, and the extents of surface irregularities and degradation were far lower than those in the control group (Figures 2 and 3).

DISCUSSION

DH is a common condition in the general population and is an increasing problem, especially in developed countries. Although people are able to delay the loss of teeth by maintaining their oral hygiene, the risk of developing DH is increasing because of various factors.[44] Today, DH is an important problem that should be emphasized in dentistry because of its increasing prevalence, negative effects on patient quality of life, and the problems it poses for oral hygiene practices.

In the present study, DDAs with different contents (TMD, GD, CWV, EN) and the Er,Cr:YSGG laser were applied to dentin surfaces alone and in combination (TMD-L, GD-L, CWV-L, EN-L). First, surface roughness changes in dentin tissue and tubule plugging effectiveness were investigated following the application of DDAs on dentin surfaces alone or in combination with laser. Then, using the erosion-abrasion cycle model, the changes in the surface roughness and tubule plugging efficiency of all of the test groups after an erosion and abrasion cycle were investigated. No significant increase in surface roughness was observed in the DDA-only groups (TMD, GD, EN, CWV) after application compared with the baseline values ($p>0.05$), whereas in the Er,Cr:YSGG laser group (L) and combined-application groups, the increases in surface roughness from baseline to after application were significant ($p<0.05$). Therefore, our first null hypothesis that DDA applications, laser application, and combination applications to the dentin surface do not significantly affect surface roughness was partially rejected.

Examination of the SEM images revealed a predominance of occluded dentin tubules in the TMD and L groups; although open dentin tubules were predominant in the EN and CWV groups, there were also closed dentin tubules in these groups. In the GD group, although there were tubule plugs and narrowed dentin tubules, no completely closed dentin tubules were apparent in the images. The increases in surface roughness in the groups treated with the Er,Cr:YSGG laser (TMD-L, GD-L, EN-L, and CWV-L) after application were significant compared with the baseline values ($p<0.05$). However, in the SEM images, more occluded dentinal tubules were observed in the combined-application groups than in the DDA-only groups.

The erosion and abrasion cycle decreases the tubule plugging effectiveness of DDAs and causes the removal of tubule plugs. Therefore, it complicates

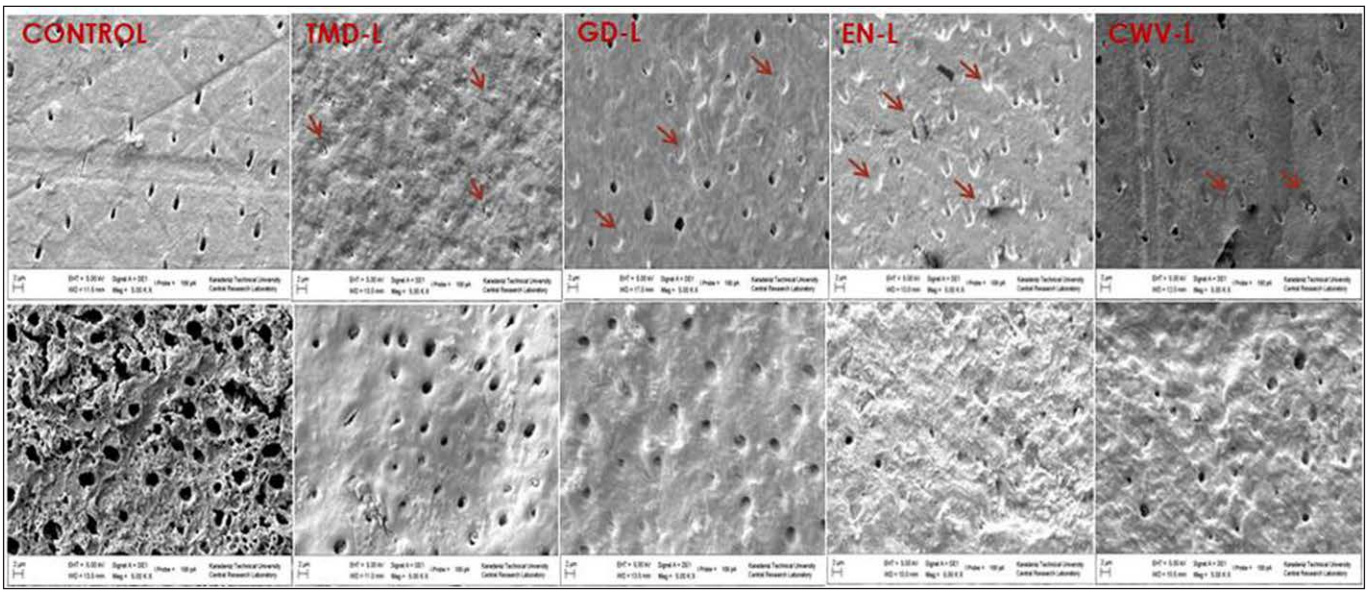

Figure 3. *SEM images of the groups of lasers combined with DDAs (the upper images were after the application groups, and the lower images were after the erosion-abrasion cycle in the same group). It was observed that occluded dentinal tubules were the majority after the applications for all of the combined groups. After the erosion-abrasion cycle, fewer irregularities were observed on the surface for all of the combined groups compared to the control group. Arrows indicate occluded dentinal tubules. CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; SEM, scanning electron microscopy; TMD, Teethmate Desensitizer.*

the effectiveness of long-term DH treatment and could cause the reemergence of DH by opening the tubules after treatment.[9,10] In addition, the changes in roughness caused by erosion and abrasion on the dentin surface can increase the risk of dental caries.[42,43] For these reasons, determining how DDA-treated dentin is affected by erosion and abrasion could provide important insight into the clinical effectiveness of DDAs.

In the present study, the increases in surface roughness after the erosion-abrasion cycle compared with after application were statistically significant in all of the groups (p<0.001). The DDA only, the Er,Cr:YSGG laser, and their combined application could not prevent an increase in roughness on the dentin surface. Therefore, our second null hypothesis that DDA-only applications, laser application, and combined DDA laser applications do not significantly affect surface roughness after an erosion-abrasion cycle was rejected.

The SEM images revealed that despite the increases in surface roughness after the erosion-abrasion cycle, the morphological changes and irregularities on the dentin surface were far fewer in all of the treatment groups than in the control group.

TMD is a calcium phosphate–based desensitizing agent. The advantage of TMD is that the supersaturation of saliva with Ca and PO_4 contributes to further HA crystal growth in the TMD layer on the tooth surface over the long term.[32,45] In the present study, while there was no significant difference in surface roughness between the TMD group and the control group after application (p>0.05), there was a significant increase after the erosion-abrasion cycle compared to the after-application measurements (p<0.001). TMD could not prevent the increase in surface roughness on the dentin surface after the erosion-abrasion cycle. SEM images of the TMD group after application showed that the dentin tubules were mostly occluded, consistent with studies in the literature.[29,46,47] In the SEM images, occluded dentinal tubules were more common in the TMD group than in the other DDA-only groups.

In an *in vitro* study by Ishihata and others, SEM images showed that all of the dentinal tubules were closed following TMD.[48] However, in an *in vitro* study by Machado and others, TMD only partially occluded the dentinal tubules. In that study, SEM images after a 5-day erosion-abrasion cycle showed that the deposits in the tubule orifices of the dentinal tubule had been removed after the cycle.[47] In contrast, in the present study, the TMD group still exhibited closed tubules after the erosion-abrasion cycle. Although TMD could not prevent the increase in surface roughness after the erosion-abrasion cycle, it reduced the surface deformation compared to that in the control group, as shown in the SEM images.

The ability of TMD to maintain tubule occlusion and reduce the formation of irregularities on the surface could be attributable to the chemical composition of

TMD. Calcium and phosphate ions dissolved from TTCP and DCPA are precipitated as HA during the setting of the material, which can form an erosion-resistant layer depending on the solubility of the HA formed. In addition, the clinical efficacy of TMD has been supported by short- and long-term follow-up studies under normal clinical conditions.[31,32,49] According to the findings of this study, TMD can maintain its tubular plugging efficacy under challenging clinical conditions such as erosion-abrasion.

GD is a desensitizing agent containing 5% glutaraldehyde and 35% HEMA. Glutaraldehyde is a biological fixative, and it has been suggested that it reacts with plasma proteins in dentin fluid and obstructs dentinal tubules.[50] In this study, a significant increase was observed in the surface roughness of the GD group after the erosion-abrasion cycle compared with the values after application ($p<0.001$), and there was no significant difference from the control group ($p>0.05$). In the SEM images of the GD group, partial tubule plugs were apparent in the dentin tubule orifices, but no dentin images showed completely occluded tubules. In addition, occluded dentinal tubules were not seen in this group after the erosion-abrasion cycle.

In an *in vitro* study, Kolker and others detected a thin layer on dentin surfaces after GD was applied but reported that most of the dentinal tubules were opened.[51] In another *in vitro* study conducted to determine the resistance of GD to acid erosion, the application of an acidic solution (Coca-Cola, pH: 3.15) after GD application on the cervical dentin surface was reported to cause the complete dissolution of the GD.[52] Another *in vitro* study revealed that open dentinal tubules were predominant in a GD group after treatment, similar to the pattern in the control group; however, SEM images revealed that the diameter of the tubules had narrowed. In that study, after a 5-day erosion-abrasion cycle, open dentinal tubules were predominant, but a layer covering the tubule orifices was observed. After the cycle, it was shown that the dentin surface with GD had lower dentin permeability than the control surface and some chemical and mechanical resistance.[53]

In the present study, GD application could not prevent the increase in surface roughness after the erosion-abrasion cycle. However, after the erosion-abrasion cycle, surface deformations and irregularities, as observed in the SEM images, were far fewer in the GD group than in the control group. These findings suggested that GD could protect the dentin surface from erosion, although there were no completely occluded dentinal tubules in GD group. Most likely, the preventive effect of GD is due to the reaction of GD with plasma proteins in the tubules. In addition, its fixative effect on dentin tissue could have protected the dentin surface from erosion.

EN is a desensitizing agent containing 5% NaF. As a result of the reaction between NaF and calcium ions, CaF_2 crystals are formed, which accumulate in the dentinal tubules.[54] Varnishes containing high concentrations of fluoride are the most widely used desensitizing products and provide highly satisfactory results in the short term after application.[55] The short-term effectiveness of fluoride varnish has been demonstrated in the literature, but its long-term results have been questioned. Saliva can dissolve CaF_2 crystals, and pain from sensitive teeth can reappear.[54-56] A 6-month clinical study showed that NaF cannot prevent DH in the long term and that DH can reappear.[57] The low effectiveness of NaF in the long term can be attributed to its insufficient adhesion to dentinal tubules and the small diameter of the CaF_2 crystals formed (approximately 0.05 μm).[57]

In our study, the SEM images of the EN-treated groups showed that the dentinal tubule orifices were generally open, but there were closed or narrowed tubules in some areas. After the erosion-abrasion cycle, most of the tubules were open, but some closed tubules remained. The surface roughness of the EN-treated specimens was not significantly different from that of the control group after the erosion-abrasion cycle ($p>0.05$). Although EN did not prevent the surface roughness increase after the erosion-abrasion cycle, it partially protected the surface, as evidenced by the SEM images showing the presence of closed dentin tubules and fewer irregularities in the EN group compared to the control group.

In a study by Alencar and others, NaF varnish was applied to the eroded surface, and surface roughness and SEM images were examined after a 3-day erosion-abrasion cycle.[8] Partial occlusions of the dentinal tubules were observed in the SEM images. However, noncontact profilometer images showed that NaF varnish was unable to fully protect the dentin surface during the erosion-abrasion cycle.[5] In contrast, Garofalo and others reported that NaF varnish can significantly reduce dentin loss, although it cannot provide significant tubular occlusion after a 5-day erosion-abrasion cycle.[26] A similar study showed that NaF varnish failed to maintain tubule plugging efficacy after a 5-day erosion-abrasion cycle.[47] In our study, the fluoride varnish was removed from the dentin surface after being maintained on the dentin surface for 6 hours, in accordance with the manufacturer's instructions; this removal could decrease the effectiveness of the varnish. In *in vitro* studies, after its application to dental hard tissues, varnish is commonly removed from the surface before

analysis. However, tubule plugs could be damaged during varnish removal from dentin surfaces.[59,60] Since fluoride varnish was not removed from the tooth surface clinically in this manner, the protective capacity of the varnish might have been increased.

CWV is a varnish containing TCP and 5% NaF. It has a higher CaF_2 precipitation potential than other varnishes because of the presence of calcium in its formula. Karlinsey and others reported that the addition of TCP to fluoride toothpaste increased fluoride retention in both enamel and dentin and facilitated remineralization.[24] Another *in vitro* study showed that the diameters of dentinal tubules narrowed significantly after CWV application.[27] In the present study, SEM images of the CWV group showed that although some of the dentinal tubule orifices were narrowed or completely occluded, most of them were open. The tubular plugging effectiveness of CWV could be reduced because of its removal from the dentin surface after waiting for 24 hours as per the manufacturer's instructions. Although the surface roughness of the CWV-applied group was not significantly different from that of the control group after the erosion-abrasion cycle ($p>0.05$), the surface irregularities observed via SEM were decreased in this group compared to the control group. In addition, in the SEM images, closed dentin tubules remained after erosion-abrasion. These findings are similar to those of Garofola and others. In their study, after erosion-abrasion cycles, closed dentinal tubules were found in CWV-applied samples, but the number of open dentinal tubules did not differ from that in the control group. In the profilometric examination, it was revealed that CWV could not protect the dentin surface against erosive wear, possibly because of its low adhesion to dentin.[26] In our study, although CWV application did not prevent the increase in surface roughness after the erosion-abrasion cycle, it enabled the dentin surfaces to be less affected by erosion.

Different types of lasers can be used in the treatment of DH. The usage of Nd:YAG and CO_2 lasers has been limited because of their thermal side effects.[38,61,62] Therefore, there has been a tendency toward the use of alternative laser types in the treatment of DH.[63] In the present study, because of the specific properties of its wavelength (2.78 μm), a medium power type Er,Cr:YSGG laser that can be used in soft and hard tissue without damaging the pulp and surrounding tissues was used. The Er,Cr:YSGG laser uses not only existing water in tissue but also exogenic water for ablation. It has been reported that exogenous water has a greater effect than endogenous water in dentin ablation.[35-37,64] Therefore, an Er,Cr:YSGG laser was used without water in this study. The results of a previous study showed that carbonization occurred even at 0.5 W when the Er,Cr:YSGG laser was used without water.[65] Since this situation can cause a rougher surface, in this study, the energy settings were chosen to be lower than the threshold at which carbonization, melting, and surface roughness could occur, so the laser was used at 0.25 W. A rougher surface can promote plaque accumulation and discoloration on tooth surfaces and increase caries risk.

The high absorption of the Er,Cr:YSGG laser emission wavelength (2.78 μm) in water causes the accumulation of insoluble salts in the dentinal tubules by evaporating the tubular fluid. It has been reported that this accumulation enables the occlusion of dentinal tubules and reduction of DH.[37,38] In the study by Gholami and others, it was shown that an Er,Cr:YSGG laser could dissolve peritubular dentin and partially or completely occlude dentinal tubules therefore reducing the symptoms of hypersensitivity in patients.[40] In the SEM images in the present study, in accordance with the literature, the dentinal tubules of Er,Cr:YSGG laser-applied samples were generally closed and narrowed, but local short cracks and irregularities were present on the surface. Depression areas were observed on interface examinations after application. The profilometric analysis results indicated that the surface roughness increased after laser application compared to the baseline level ($p<0.05$).

SEM images of the Er,Cr:YSGG laser group after the erosion-abrasion cycle showed that closed dentin tubules were in the majority. In addition, local short cracks and irregularities were rarely seen on dentin surfaces and were no more common than they were after application, and degradation and irregularities on the surface were less common in this group than in the control group. In addition, after the erosion-abrasion cycle compared to after application, there was a significant increase in the surface roughness values of the laser group ($p<0.001$). Additionally, after the erosion-abrasion cycle, the surface roughness of the laser group was greater than that of all of the DDA and combination DDA-laser groups except GD-L.

The effectiveness of laser application can be affected by many factors, such as laser wavelength, energy output, and dentin surface conditions (dry or wet surface). Laser application can increase surface roughness as well as cause dentinal tubule occlusion, with ablative and dissolving effects on dentin tissue. Therefore, cracks and irregularities can occur on the dentin surface. These cracks can render the dentin surface more susceptible to erosion and abrasion and cause a significant increase in surface roughness

after the cycle. In addition, although laser application increased the surface roughness of dentin surfaces after the cycle, dentinal tubule plugs were still widely observed, and the degradation and irregularities on the surface were far less widespread than those in the control group. Although the surface roughness was increased by laser application, the tubule plugs formed by the laser resisted erosion-abrasion. These findings show that lasers can be effective for DH treatment in difficult oral conditions.

In the present study, in addition to its use alone, the Er,Cr:YSGG laser was used in combination with DDAs, and tubular plugging efficiencies and surface roughness were investigated. Compared to those in the DDA-only groups, occluded dentin tubules were more abundant in the groups treated with DDA in combination with laser. In addition, in the combination DDA and laser groups, tubule plugging activity persisted after the erosion-abrasion cycle, as shown in the SEM images. Consistent with the literature, this study indicates that the combination of DDA treatment with laser irradiation is significantly more effective in dentinal tubule occlusion than DDA alone.[57,66]

In an *in vitro* study, TMD and GD in combination with Er:YAG was found to be more advantageous than laser or DDAs alone. The occlusion rates of dentinal tubules were higher in the DDA-laser combination groups. Most dentinal tubule occlusions were observed under laser application combined with GD. However, the application of GD or TMD alone did not damage the dentin surface, unlike laser treatment. As shown by atomic force microscopy observations, the groups treated with DDA in combination with Er:YAG laser showed a very rough surface characterized by grooves, and prominent cracks and craters were apparent in the dentinal tubules.[28]

Different studies have suggested that laser applications in combination with fluoride can increase the effect of fluoride.[57,67,68] In an *in vitro* study conducted to analyze the mechanism of the combined application of laser and fluoride, fluoride penetration in the root dentin was found to be better than that with fluoride alone and to inhibit demineralization.[67] In another *in vitro* study, the combined use of CWV and Nd:YAG lasers was reported to result in a surface structure in which most of the dentinal tubules were occluded.[27]

In this study, after the erosion-abrasion cycle, the surface roughness values of the TMD-L, EN-L, and CWV-L groups were found to be significantly lower than those of the Er,Cr:YSGG laser-only group ($p<0.001$). The reason for this outcome might be that the layer formed by DDAs before laser application reduced the negative effects of the laser on the dentin surface.

In addition, the tubule plugs formed by the combined-application groups were resistant to the erosion-abrasion cycle, similar to those of the laser group, and there was less surface damage in these groups than in the control groups. These findings suggest that combined DDA-laser treatment could be more effective for DH treatment under different oral conditions than DDA treatment alone. Laser application increased the tubular plugging efficiency of DDAs. In addition, resistant tubule plugs after the erosion-abrasion cycle could serve as a barrier against bacteria, preventing bacteria adhering to the dentin surface from progressing to the dentin tubules.

One of the limitations of this *in vitro* study was that although the early term results of DDAs and laser applications were determined by both SEM examinations and profilometric analyses, long-term effects were not examined. In addition, artificial saliva containing calcium was used in our study. Artificial saliva cannot show the enzymatic and microbiological effects of human saliva. Human saliva can protect tooth surfaces against erosion and abrasion by forming pellicles on tooth surfaces in the oral environment. In addition, the use of artificial saliva could have increased the effects of the tested DDAs because of its calcium content.[26]

However, the different findings of *in vitro* studies are due to differences in the large number of experimental parameters, such as the type of DDA; the type of laser; the laser application parameters, with different effects on tissue; the pH of the acidic solution used for erosion; test cycle time; and other various assessment methods.

In addition, in this study, since test specimens obtained from teeth extracted for orthodontic purposes were used, the detected changes in surface roughness and SEM properties are likely smaller than those that occur in teeth with clinical DH. Dentin surfaces associated with clinical DH complaints and frequent exposure to erosion abrasion could be expected to be more affected and damaged than the teeth observed here, especially in cases in which the buffering effect of saliva is weak. Further damage could be expected on clinically sensitive dentinal surfaces and areas frequently exposed to erosion abrasion, particularly where the buffering effect of saliva is weak. Therefore, more comprehensive *in vitro* and clinical studies are needed to gain insight into this issue.

CONCLUSIONS

Effects of different DDAs, Er,Cr:YSGG laser, and their combined application on dentin surface roughness, their effectiveness in occluding dentin tubules, and the resistance of these applications to erosion-abrasion

cycles were investigated. After application, DDAs alone did not cause an increase in the surface roughness of dentin, whereas all other treatments led to provoked surface roughness. In addition, none of the applications could prevent an increase in surface roughness after an erosion-abrasion cycle. Despite the increase in surface roughness of all test groups, morphological changes, cracks, and surface irregularities on dentin surfaces were less apparent. In addition, laser and DDA treatment increased the plugging efficiency of DDAs resulting in tubule plugs more resistant to erosion-abrasion cycle. The findings suggest that combined laser and DDA treatments could be more effective than DDAs alone.

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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 Karadeniz Technical University, Schoolof Medicine. The approval code issued for this study is 2019/60.

Conflicts 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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