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In Memoriam

D. Tucker is a man with a documented CV more than four pages long. Add to that the countless honors and awards he has received over the years, and you could write a book! I want to take this opportunity to encourage you all to take a more personal look at this great man.

Dr Richard Vaughn Tucker was born in Orofino, Idaho, on November 25, 1922. He attended the University of Washington from 1940 to 1942, where he met Elaine, whom he described to me as "the love of my life." They continued on to St Louis, Missouri, where Dick attended the Washington University School of Dentistry, and they were married in 1944. Dick served in the US Navy from 1946 to 1948. After his Naval service, he practiced briefly in Seattle, but he told me not long ago that Seattle was too big and busy for his liking. When one of his patients told him of Ferndale, a small farming community of 500, north of Bellingham, that was in need of a dentist, he investigated. He found it was exactly the kind of town where he wanted to practice, and Elaine thought it was a perfect place to raise a family. Dick would practice there until he retired at age 91. Dick and Elaine were married for 71 years and had 4 children: Ann Marie, Dick Jr, Victoria, and Martha. Dr Tucker's passion for dentistry was so contagious that Vickie and Dick Jr became dentists, and Vickie also married a dentist, as did her sister Anne Marie. Can you imagine sitting around the dinner table at Christmas or Thanksgiving in the Tucker family home?

It was in Ferndale, WA, that Dr Tucker would meet his dentist, first mentor, and the man who would become his good friend, Dr George Ellsperman. George was a large man, both in stature and operative reputation. It was from George that Dick coined the phrase "Don't go chippy on yourself." Dr Ellsperman was also one of the 12 original members mentored by "The Father of Gold Foil," Dr W. I. Ferrier.

Dr Ellsperman restored Dick's mouth in gold, one inlay at a time. Until the day he retired, Dick would gladly have any dentist inspect the beautiful work that George had done, none of which had failed in more than 60 years.

Dr Tucker was so impressed by the quality of Dr Ellsperman's work that he wanted to show George

some of his own best work. After completing four quadrants of beautifully carved and polished alloys on one of his patients, he paid the man a whopping five dollars to travel to Dr Ellsperman's office for inspection. George said nothing to Dick about the quality of the work. His only comment to him was that he was wasting his talent doing alloys, and if he wanted his work to last, he should start doing gold castings. Dr Elsperman encouraged Dick to start doing gold foil restorations. However, George was so impressed with the quality of Dr Tucker's restorations that when a position became available in the Vancouver Ferrier Gold Foil Study Club, mentored by Dr Gerry Stibbs, George presented Dick as their newest member, without audition. In those days, the Ferrier Study Clubs were among the most elite, and membership was based on operative ability. Acceptance of a new member was based on the quality of your operation during your audition.

Despite the tremendous pressure he must have felt, Dick flourished in this new environment.

Over the years, Dick recognized that he shared the same fundamental values as Dr Gerry Stibbs, and they became the very best of friends.

During this period, Dr Tucker continued to perfect his gold casting technique. Recognizing that the finest margin was that of a gold foil, he strived to achieve that in his casting and finishing techniques.

Over time, as Dr Tucker would bring his patients to the Gold Foil Study Club, many members would take note of and comment on the beautiful gold cast restorations that they could see next to his excellent foils. A fellow study club member known for his excellent gold castings, Dr Joe Zokol, was so impressed with the fit and finish of Dr Tucker's beautiful castings that he encouraged him to mentor a Cast Gold Study Club in his Vancouver office. Joe and Dick agreed that they would imitate the format of the Ferrier Study Clubs: monthly lectures and operations, followed by critiques and a dinner with refreshments, but they would use the guidelines of armamentarium, preparation design, and finishing techniques developed by Dr Tucker. Dick's artistic genius was clearly evident in his unique preparation designs and his extremely efficient use of a minimal number of burs and hand instruments.

On January 8, 1976, the R. V. Tucker Academy was born in the office of Drs Joe and son, Ron, Zokol. By good fortune, my long-time friend, Dr Laurie Vanzella, and I were invited to be members of this new study club. We had no idea at the time, that we had literally won the Power Ball Lottery of operative dentistry! We were at the start of the most amazing and fulfilling journey of our professional careers.

On that day, Dr Tucker gave two demonstrations. In the morning, he prepared five gold inlays, took impressions, and temporized the quadrant in less than 2.5 hours. After lunch, he seated, finished, and polished a second quadrant of four gold inlays in less than two hours. No one could believe how fast he was able to prepare and finish these two cases, especially when the finished afternoon case was the most beautifully finished work we'd ever seen. He made it look effortless. When we asked him how he was able to operate with such speed, he explained that each operation was a series of steps. The key was to do each step as perfectly as possible before moving on to the next step, eliminating the need to go back. He told us over and over that the key to success was to focus on learning to cut the preparations as perfectly as possible, and over time, with repetition, the speed would come. Perfection first, speed second. Over the years, we found that this Tucker philosophy applied to all aspects of dentistry and would enable us to become much better operators in other fields of dentistry.

Dr Tucker felt that the social aspect of our study club was vitally important. Our appreciation for excellence in dentistry was matched by our appreciation for fine dining. It was at these dinners where our friendships grew, and we felt comfortable to speak frankly about other issues related to dentistry. At Christmas time, we would dress up in our tuxedos and have a grand dinner and fine wines; this was one of Dr Tucker's favorite traditions.

Our annual Whistler ski weekend was another tradition unique to our group. Apre ski consisted of great dinners, lots of great wine, games of bridge, and Dick's favorite, Honest Farmer, which he never seemed to lose at. Dick's "fireside chat" on investment principles and strategies was a part of the weekend we always looked forward to. We nicknamed him "the Warren Buffett of dentistry."

As his techniques gained notoriety and the demand for him to lecture at various universities and dental meetings grew, he inspired other young dentists to start Tucker Study Clubs of their own. The R. V. Tucker Academy was gaining worldwide recognition. In 1986, Dr Tucker introduced the idea of an associated meeting for all existing study clubs to gather once a year. The first meeting was held in the Olympic Hotel in Seattle and at that time consisted of lectures and social activities only. Two years later, club 1 hosted the Tucker Associated meeting in Vancouver, BC, Canada, and it was at that meeting where the George Ellsperman lecture and clinical operations were added to the programs structure. When the demand for study clubs in Italy and Germany arose, Dick decided it was time to have some of his founding members help mentor these new groups. Dr Tucker took club 1 to Italy and later club 3 to Germany to present a Tucker Technique program and assist in mentoring. It was a very special time for us, as we really recognized how much Dick had taught us and how much of that knowledge we could share with our new friends in Europe. Over the years, club 1 and club 3 made other trips back to Italy and Germany to mentor and established lifelong friendships with the Italian and German study clubs. It was Dick's idea that

whenever club 1 mentored in Italy or Hawaii, we should spend a few days together socially after our mentoring duties were over. This really enriched our experience and confirmed to all that Dr Tucker practiced what he preached in all aspects of his life.

With the help of Dr Dennis Miya, Dick developed the Tucker Institute. This was a 1-week summer program held at the University of Washington, modeled after the program that Dick started years earlier at the University of British Columbia, where dentists were introduced to the Tucker Technique through lectures and operations. As dentists were exposed to these techniques, the demand for new study clubs grew exponentially. Dr Tucker developed a new protocol whereby members of the six founding study clubs would have the opportunity to mentor a new study club. Dr Tucker's schedule was more demanding than ever, yet he still found time to attend the first meeting of a newly formed study club to lecture and often operate. He kept up this schedule until 2007, when he, in his own words, "started to slack off," only continuing to mentor the Vancouver Ferrier Gold Foil club plus three Tucker Study clubs thereafter. I've calculated a rough estimate of how many hours he dedicated to study club mentoring and lecturing, and it is in excess of 12,000 hours, which was close to six years of his life between 1976 and 2013. This was on top of maintaining a full-time dental practice in Ferndale. During the last decade, he began to limit his study club activities further to be home with Elaine each night.

In September 2009, the R.V. Tucker Associated meeting was held in Vancouver, BC, Canada. Dr Tucker was presented with a hand-carved Squamish Nation "talking stick." The Squamish elders use the talking stick to teach the young people how to interact in a respectful manner with others. In their culture, they teach never look up or down at someone but to look across at them, as everyone is equal. This made the talking stick such a meaningful gift for Dick, as it really symbolized his own teaching and mentoring style. I believe this is truly the cornerstone of Dr Tucker's genius. As Dick's well-deserved reputation continued to grow, he remained always humble, gracious, and approachable. He never looked down on anyone. Regardless of a dentist's educational or operative background, Dick had a magical ability to encourage the very best in us. His gentle way, how he would ask permission to show you something that would help, and his quiet strength inspired us not only to do our very best in dentistry, but also in our lives.

On May 6, 2011, Dr Tucker gave his last presentation on conservative cast gold restorations at the University of British Columbia. His instructions to me were simple: tuition should be just enough to cover the costs so that as many dentists as possible could attend, and he refused to take an honorarium. Because this would be his last lecture, he would bring his entire staff and asked me if I would be so kind as to introduce each one of them in my opening remarks as a way of honoring their contribution to his success. The program sold out. When Dr Tucker concluded his presentation, the audience gave him a standing ovation.

In November 2012, more than 200 of Dr Tucker's family, friends, fellow academy members, and staff attended a surprise party for his 90th birthday in Bellingham, WA. Dick knew only of a presentation from the Hawaiian clubs 44 and 50 to club 1, and Dr Tucker was honoring their longstanding relationship as the first mentors for the Hawaiians. They presented Dr Tucker and all members of club 1 with a silver goblet that they would all drink together with the Hawaiians whenever they met to toast this special friendship. Then into the next room for lunch and—SURPRISE! It was a night to remember. We all thought he might just live forever. However, over the next year when he would travel to Vancouver to mentor club 1, he would often remark that it was time to retire as "no one should see a dentist over 90 years of age!"

As his health began to decline, Dick finally retired. He rallied enough to attend the R. V. Tucker Associated meeting in Honolulu in August 2014 due to the gracious care of his laboratory technician and personal assistant Luba. She would stay on with Dr Tucker as his personal assistant until his last breath. She is a true angel, and we are so grateful that she cared for him like her own father.

Some of you may know how much Dick loved to sail. He treasured time out on their beautiful sailboat, Line Angle. He had so many happy memories related to family and friends out on the water sailing with him. I've never seen him look happier or more at peace than when he was sailing the Line Angle in a good wind. On June 1, 2015, the wind was taken from his sails when his beloved Elaine passed away.

He never truly recovered from this.

On December 23, 2015, Nancy and I met Dick Jr, Luba, and Dick Sr at his favorite Italian restaurant in Bellingham Bay for a Christmas lunch. He graciously signed four of the club 1 Tucker

anthology books as gifts for the founding members of the Italian study clubs: still thinking of others and making a contribution. This would be the very last time I would see my dearest friend and mentor before he went to be with his Elaine. On the morning of January 12, 2016, at age 93, Dr Richard Vaughn Tucker passed away peacefully.

All the years that Dr Tucker practiced in Ferndale, a plaque hung in his main operatory that said "It's not what you think that counts, It's not what you believe that counts, It's not what you say that counts, It's what you do that counts!" Winston Churchill once said that a man's wealth and life is not measured by what he gets, but by what he gives. For me, Dr Tucker was the wealthiest man I have ever met. I will miss him beyond measure. In the words from our dear Hawaiian friends, written on his goblet, which will now be turned over to read "Pau Hana," the work is done, it's time to rest, we've done our best.

Respectfully submitted,

Terry McKay

January 17, 2015

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Clinical Technique/Case Report

Ceramic Veneers and Direct-Composite Cases of Amelogenesis Imperfecta Rehabilitation

S Shibata • CMC Taguchi • R Gondo
SC Stolf • LN Baratieri

Clinical Relevance

Amelogenesis imperfecta is a hereditary disease affecting the quality and quantity of enamel. Patients usually suffer from oral complications and poor dental esthetics, which directly affect their quality of life. Function and esthetics can be restored with different restorative materials, such as ceramic and composite resin. Dentists need to be aware of the best material to use for each patient.

SUMMARY

The aim of this article is to present two case reports for the treatment of patients affected with amelogenesis imperfecta. One case was treated with composite resin and the other case with ceramic veneers. Esthetic and functional results were achieved using both treatments, and a review of advantages and disadvantages is presented.

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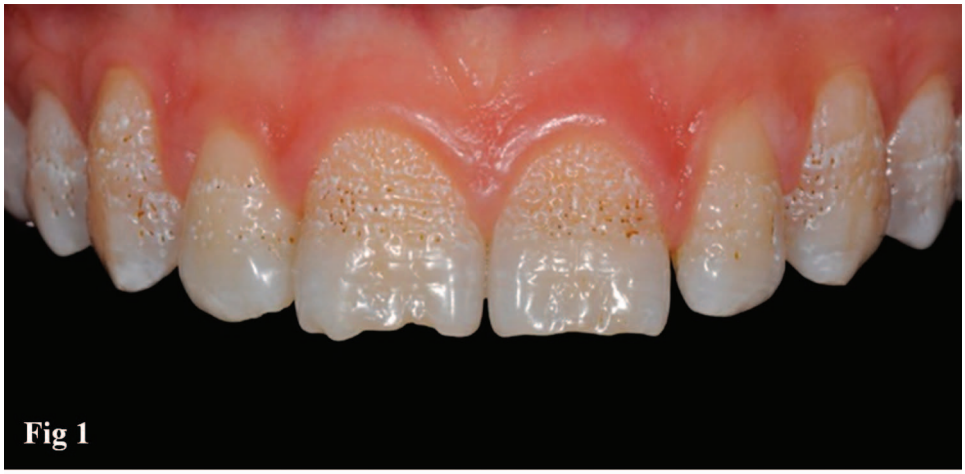
INTRODUCTION

Amelogenesis imperfecta (AI) is a term for a clinically and genetically heterogeneous group of conditions that are caused by mutations in a variety of genes that are critical for normal enamel formation. The gene mutations alter the quality and/or quantity of enamel in the primary and permanent dentitions. Initial classifications of AI had been based exclusively on the phenotype (appearance). More recent classifications include both the phenotype and the mode of inheritance. The outdated AI classification system recognized four phenotypes: 1) hypoplastic, 2) hypomaturation, 3) hypocalcified, and 4) hypoplastic-hypomaturation. However, today, at least 14 AI subtypes are identified when both phenotype and mode of inheritance are considered.[1-3]

AI prevalence has been reported to vary from 1/700 to 1/14,000, depending on the population



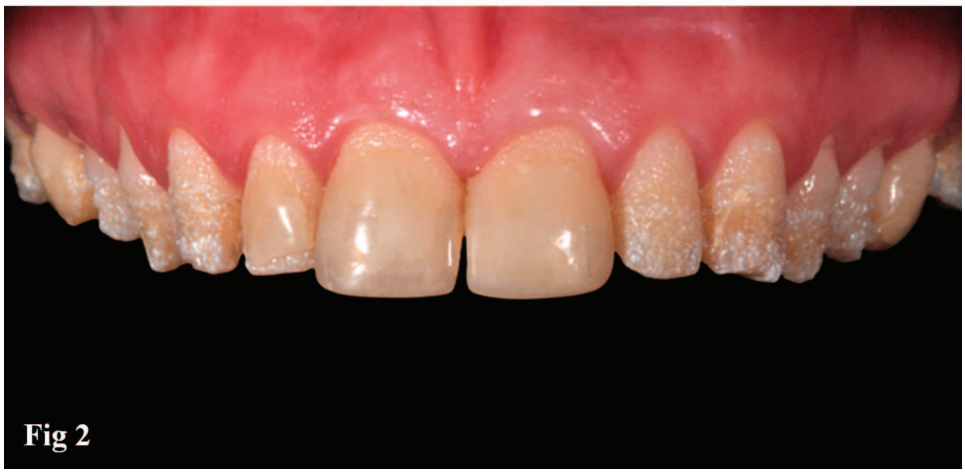


Figure 1. *Initial view of Case 1.*
Figure 2. *Initial view of Case 2.*

studied. AI affects all tooth enamel of the affected individuals, without reference to chronology and occasionally in association with other generalized conditions. Based on the literature, regardless of AI subtype, patients have similar oral complications and poor dental esthetics. For all patients, the affected teeth may be discolored, sensitive, or prone to either preeruption or posteruption disintegration.[3,4]

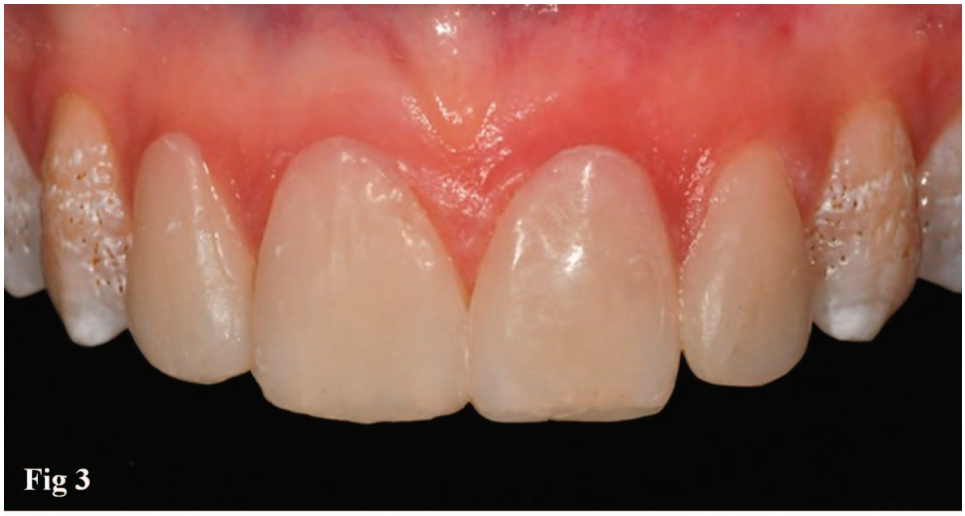
This developmental dental anomaly appears to have a profound impact on patients' quality of life. Hashem and others[5] studied the impact of hypodontia and AI on the quality of life and self-esteem of adult patients. For AI patients, the condition significantly affected psychological discomfort related to physical, psychological, and social disabilities. Although different treatment modalities have been described for the rehabilitation of AI in adults and children, treatment is always a great challenge to clinicians.

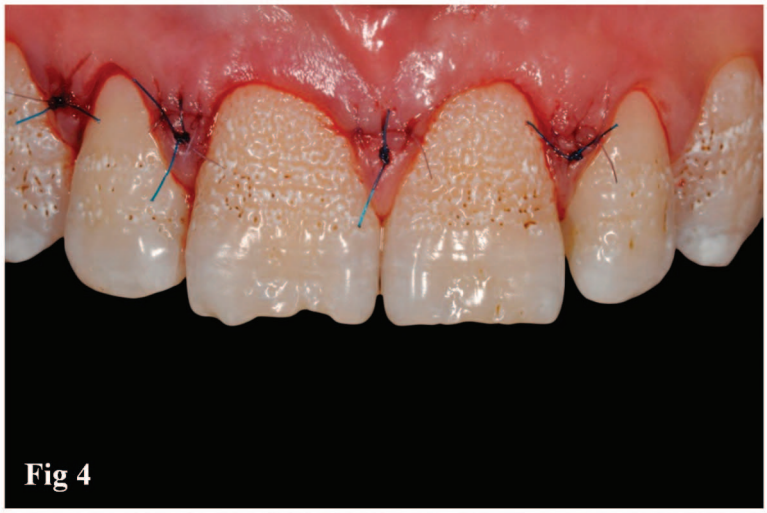
The aim of this article is to describe minimally invasive techniques for the prosthetic rehabilitation of two young adult female patients with AI. This was based on conservative and adhesive treatments through the use of laminate veneers and direct composite resins.

CLINICAL CASE REPORT

In both cases reported, female patients, 17 and 19 years old, were diagnosed with the hypoplastic type of AI (Figures 1 and 2). Both family histories revealed that the patients' sisters also had similar dental deformities. Clinical examination revealed porous enamel, with generalized mottled and chipped appearance, and generalized discoloration of all teeth (posterior and anterior). The enamel layer could be distinguished from the underlying dentin; however, it was generally thin. Radiographic examination with panoramic and periapical x-rays did not reveal any missing teeth or periapical lesions. Both patients were dissatisfied with their dental appearance.

Treatment goals were to prevent further tooth destruction, improve esthetics, and restore oral





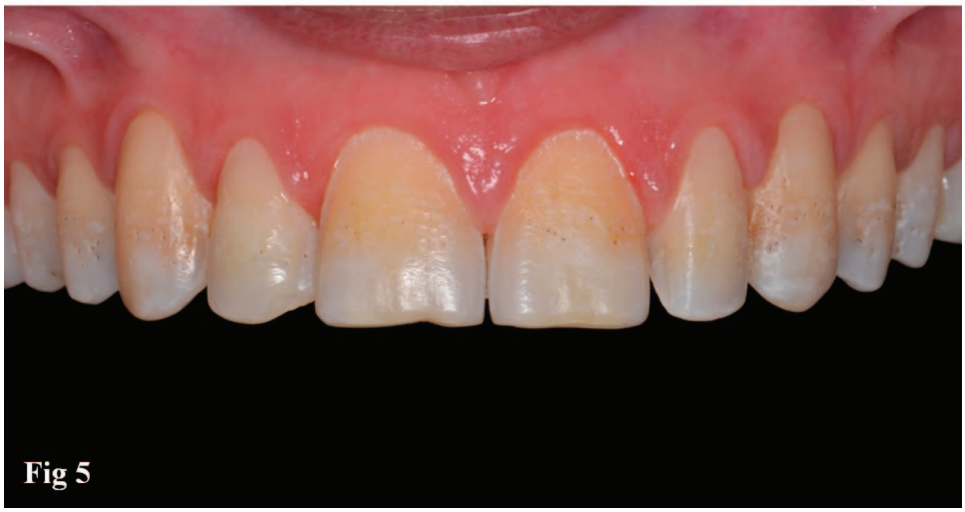
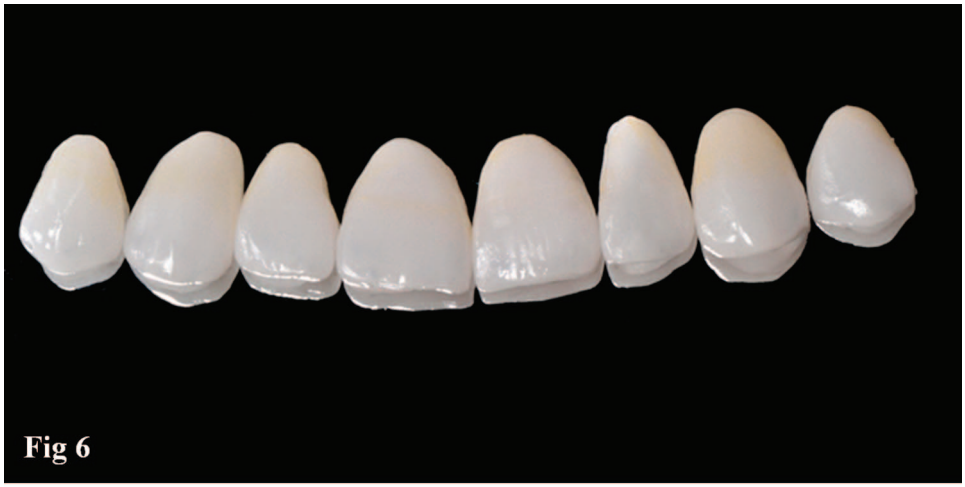


Figure 3. *Case 1: Composite resin mock-up.*
Figure 4. *Case 1: Gingivectomy in anterior region.*
Figure 5. *Case 1: Teeth after all preparation.*

function. Initial impressions were obtained and study casts were constructed with hard stone. A full wax-up was performed on the study casts, and a direct mock-up was carried out in the patient's mouth with an auto-mixing, self-curing bis-acrylic resin (Protemp Plus). After checking the occlusion, both patients approved the treatment plans, which are as follows for each case.



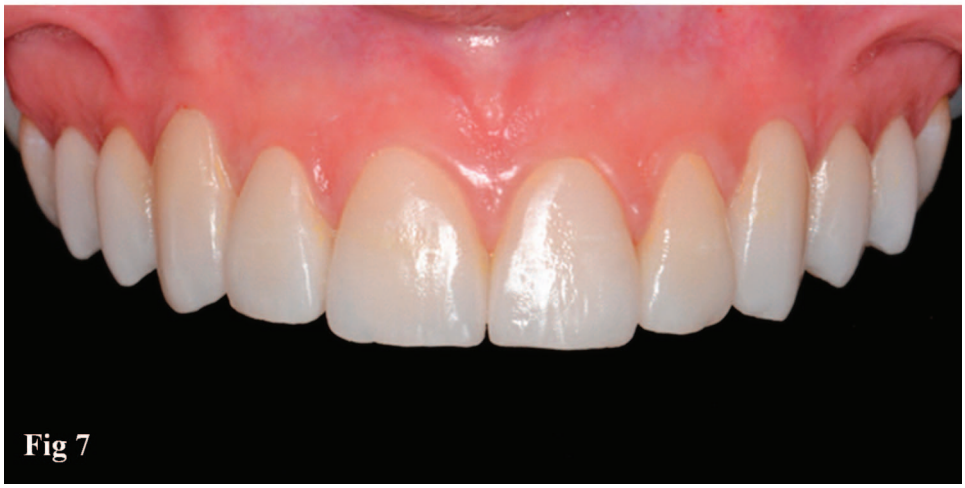


Figure 6. *Case 1: Ceramic laminate veneers.*
Figure 7. *Case 1: Final clinical result.*

Case 1: 17-Year-Old Female

- Periodontal treatment (gingivectomy)
- Preparation of maxillary anterior teeth (Nos. 4-13) for ceramic laminate veneers
- Fabrication of laminate veneers
- Adhesive cementation

Initially, periodontal surgery was carried out using a composite resin mock-up as a guide (Figure 3). A gingivectomy was sufficient to achieve correction of gingival levels and proper width-to-length tooth ratios (Figure 4). Three months after the surgery, the maxillary teeth were prepared with a diamond bur No. 2135, under water spray. All preparation had less than a 0.5-mm depth, and the margins were placed on sound enamel (Figure 5). Final impressions and occlusal registrations were obtained with polyvinyl siloxane elastomer material, and provisional restorations were made from composite resin (Empress Direct) without previous enamel etching (Table 1).

All-ceramic laminate veneers were fabricated with a lithium disilicate–reinforced ceramic (IPS e-max Press; Figure 6). The internal surfaces of the ceramic restorations were etched with 5% hydrofluoric acid for 20 seconds, rinsed with water, and dried with an air spray. One layer of silane (Monobond-S) was applied for 60 seconds on the etched surface and dried for 60 seconds. The enamel surfaces were etched with 37% phosphoric acid for 20 seconds. After being rinsed and dried, two layers of an adhesive (Ambar) and mild air jets were applied until a shiny appearance was observed on the uncured surface. A small amount of photo-cured resin cement (Variolink Veneer) was applied over the restoration's internal surface and positioned. After the excess was removed, the resin was light-cured for 60 seconds using an LED unit (900 mW/cm^2 output). Finishing and polishing of the margins were carried out, and the occlusion was checked (Figure 7).

The patient was satisfied and examined two weeks later. All restorations were intact, oral hygiene was

Table 1: *Materials Used*

	Case 1
Protemp Plus (bis-acryl temporally resin)	3M ESPE (St Paul, MN, USA)
KG #2135 (diamond bur)	KG Sorensen (Cotia, Brazil)
Express XT (polyvinyl siloxane)	3M ESPE (St Paul, MN, USA)
Empress Direct (composite resin)	Ivoclar Vivadent (São Paulo, Brazil)
IPS e-max Press (Lithium disilicate-reinforced ceramic)	Ivoclar Vivadent (São Paulo, Brazil)
5% Hydrofluoric acid (porcelain etching gel)	FGM (Joinville, Brazil)
Phosphoric acid 37% (etching gel)	BM4 (Florianópolis, SC, Brazil)
Monobond-S (silane agent)	Ivoclar Vivadent (São Paulo, Brazil)
Ambar (adhesive)	FGM (Joinville, Brazil)
Variolink Veneer (resin cement)	Ivoclar Vivadent (São Paulo, Brazil
Bluephase (LED unit)	Ivoclar Vivadent (São Paulo, Brazil)
Case 2	
Protemp Plus (bis-acryl tempory resin)	3M ESPE (St Paul, MN, USA)
Phosphoric acid 37% (etching gel)	BM4 (Florianópolis, SC, Brazil)
Single Bond2 (adhesive)	3M ESPE (St. Paul, MN, USA)
Empress Direct (composite resin)	Ivoclar Vivadent (São Paulo, Brazil)
Elite Glass (polyvinyl siloxane)	Zhermack (Badia Polesine, Italy)
Bluephase (LED unit)	Ivoclar Vivadent (São Paulo, Brazil)
Polyester Strip Matrix	TDV (Santa Catarina, Brazil)
KG#9642FF (diamond bur)	KG Sorensen (Cotia, Brazil)
Soflex (polishing discs)	3M ESPE (St. Paul, MN, USA)
Astrobrush (impregnated brush)	Ivoclar Vivadent (São Paulo, Brazil)

maintained, and gingiva appeared healthy with no inflammation or recession.

Case 2: 19-Year-Old Female

- Removal of old restorations
- Direct restoration with composite resin

Initially, old restorations were removed with diamond burs and a scalpel blade. The restorations were carefully removed to preserve sound enamel and avoid any type of preparation. Afterward, the hypoplastic enamel was sandblasted with aluminum oxide particles to remove composite remains and debris from the surface (Figure 8).

The anterior incisors were restored, one by one, respecting the following protocol: enamel surface etched with phosphoric acid for 30 seconds, rinsed with air/water spray for the same period, dried for 60 seconds, two layers of adhesive (Single Bond2) applied and light-cured for 15 seconds.

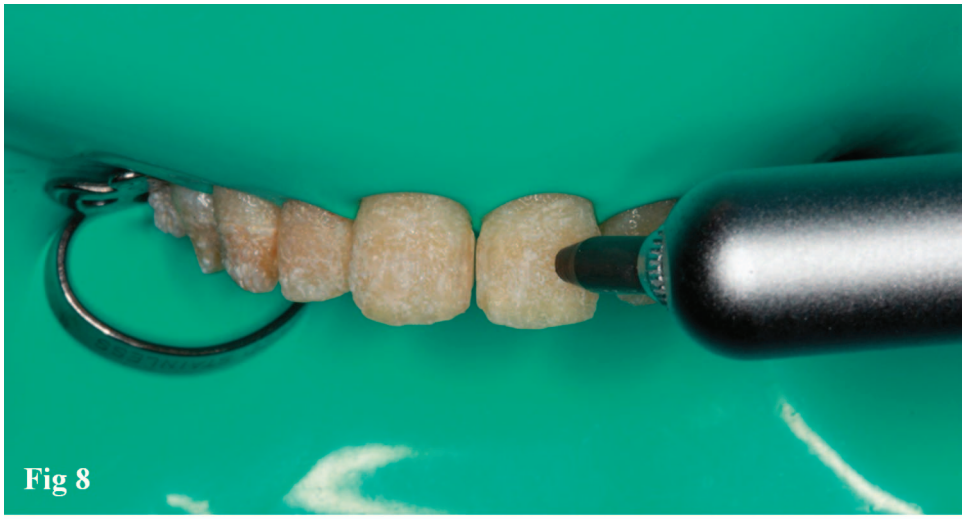
All restorations were performed using an auxiliary lingual index to determine the incisal edge. An initial layer of shade BL-L enamel composite resin was used and light-cured for 60 seconds (Figure 9). To enhance restoration value, a composite layer shade A2 was inserted and light-cured for 60 seconds (Figure 10). A transparent index made from polyvinyl siloxane (Elite Glass) had been fabricated to record the facial surface of the wax-up (Figures 11 and 12). Then, a final composite layer of enamel resin, shade BL-L, was inserted with the aid of the index (Figures 13 and 14). Light-curing was performed through the index for 60 seconds and final polymerization for a further 60 seconds without the index (Figures 15 and 16). While in the proximal areas, the restorations were performed by a pull-through technique. One increment of enamel resin, shade BL-L, was pulled from the facial toward the lingual surface with a celluloid strip (TDV). The excess of material was removed with scalpel blade No. 12 and diamond burs FF.

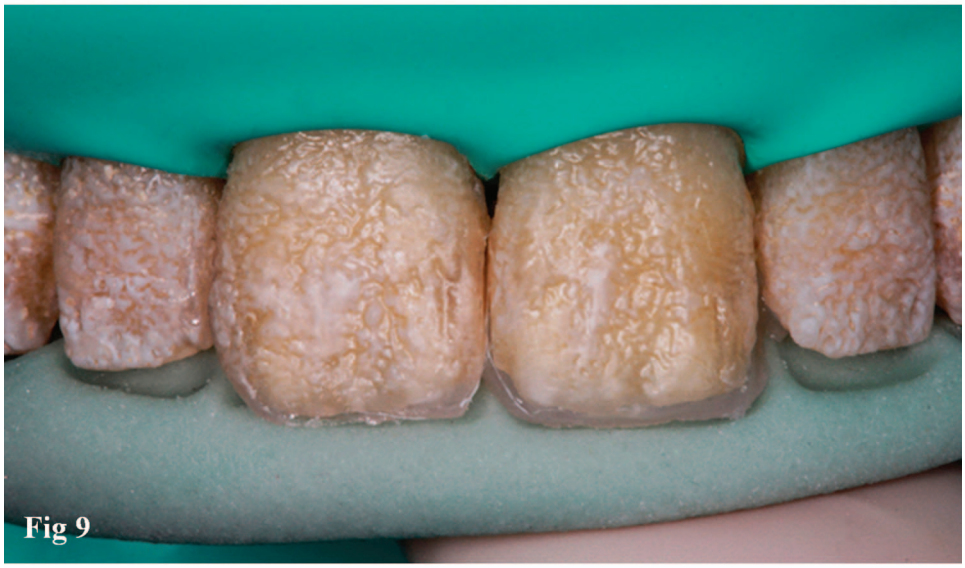
After 24 hours, finishing and polishing of the restorations were performed with diamond burs No. 9642FF (KG Sorensen), abrasive discs of different grades (Sof-lex), and a brush impregnated with silicon carbide (Astrobrush; Figure 17).

DISCUSSION

AI affects the quality and/or quantity of enamel in the primary and permanent dentitions. Both cases showed affected enamel, which was easily distinguished from dentin. Although in the first case the enamel surface had not been equally affected by a mottled appearance, a thin layer and incisal edge fracture were observed. The enamel severity depends on the gene mutation, which defines different AI phenotypes.[1,3]

Restoring esthetics and function of a young patient with AI is a challenge for the clinician. The treatment options vary considerably, depending mainly on the patient's age, AI type, disorder severity, and intraoral situation.[6,7] Treatment options advocated in the literature include composite resins, stainless steel crowns, all-ceramic





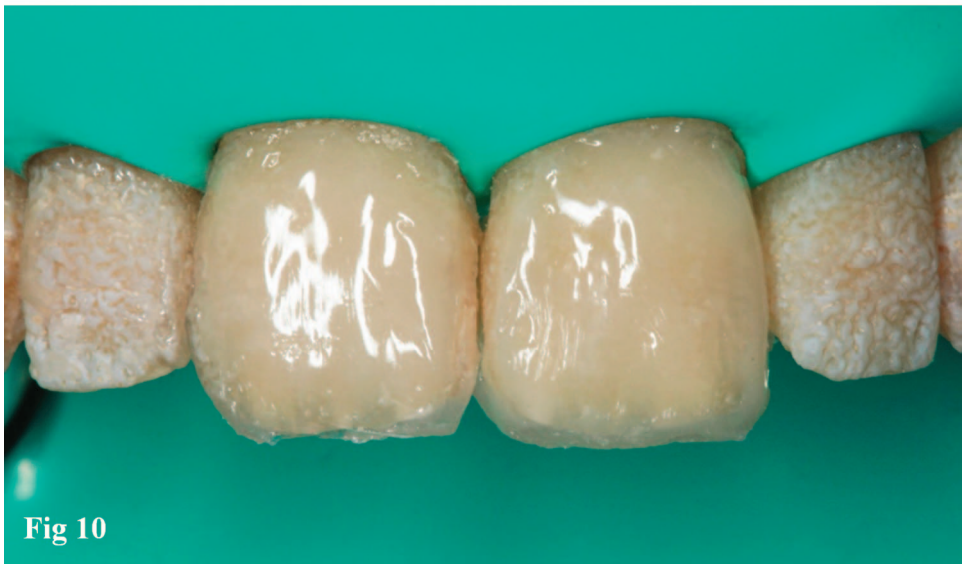
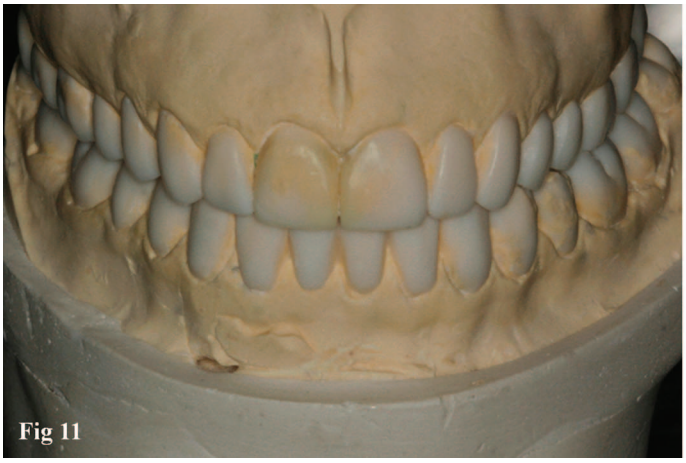


Figure 8. *Case 2: Sandblasting with aluminum oxide particles.*
Figure 9. *Case 2: Composite resin palatal enamel layer.*
Figure 10. *Case 2: Composite resin dentin layer.*



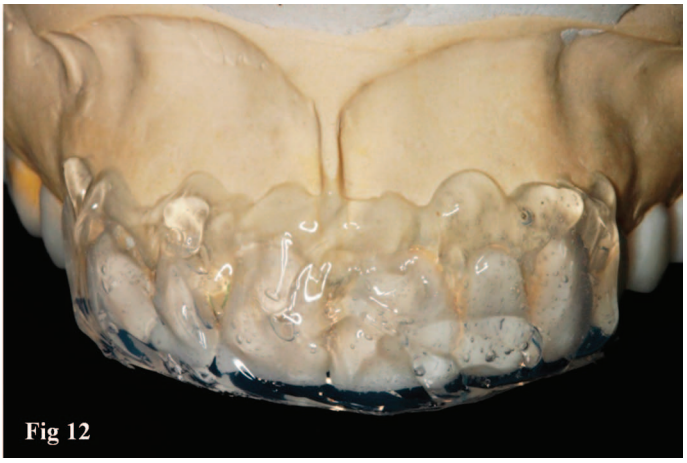


Figure 11. *Case 2: Additive diagnostic wax-up.*
Figure 12. *Case 2: Transparent index fabricated.*

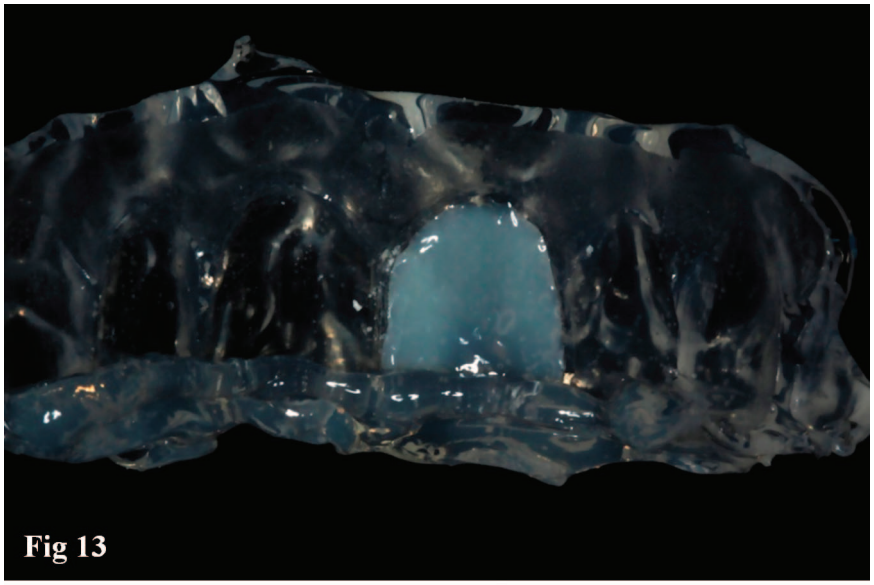
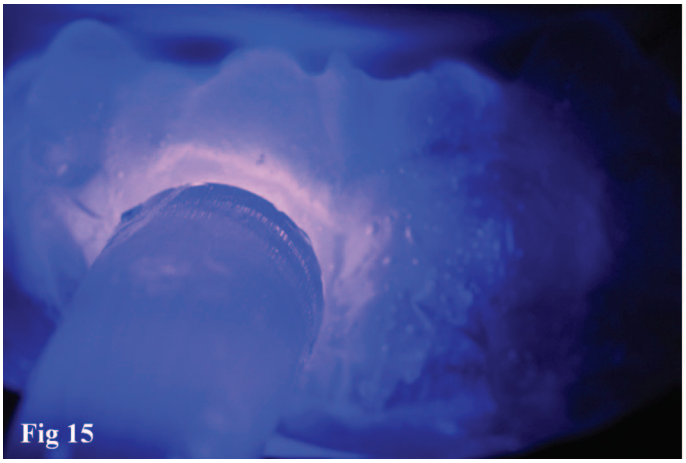


Figure 13. *Case 2: Enamel composite inserted in the internal surface of the index.*
Figure 14. *Case 2: Index in position.*



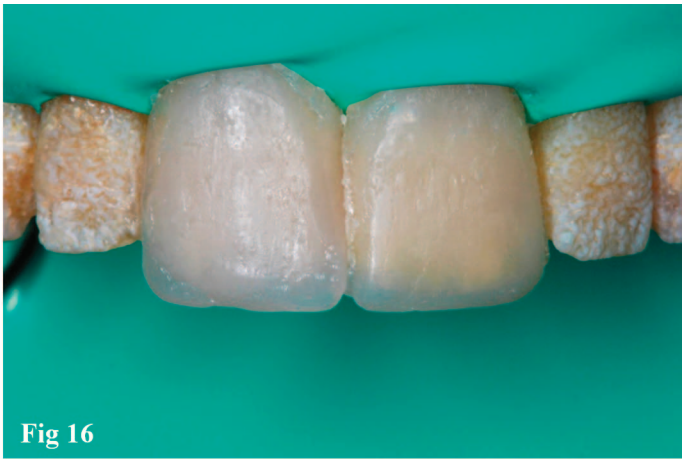


Figure 15. *Case 2: Composite resin enamel photoactivation through the silicone index.*
Figure 16. *Case 2: Vestibular composite resin enamel layer.*

crowns, and currently, the more frequently used laminate veneers.[2,3] In this clinical report, two young patients were diagnosed with hypoplastic type AI but were rehabilitated with different restorative materials since the esthetic demand was not the same.

Conventional crowns are the most common treatment recommended for patients with AI.[8,9] Most patients, consequently, have a large amount of healthy tooth structure removed. Optimal preparation design for ceramic crowns is a paradox, since patients already suffer from tooth-tissue loss and pulpal injury, especially young patients.[10] The decision to remove all enamel or keep an enamel layer depends on the depth and extent of the lesions. The clinical appearance of the enamel during tooth preparation plays a decisive role.[7] Several studies have illustrated the use of all-ceramic crowns,[4,10,11] but other authors have described less invasive treatments, including composite resin and laminate veneers.[9,12,13]

Composite resin is able to mimic tooth color through anatomical stratification and proper placement of tints and opaquers, to enhance the esthetic value. The long-term success of direct composites may depend on patient selection, cavity location and size, material choice, and operative technique. Risks for failure include fracture and partial loss

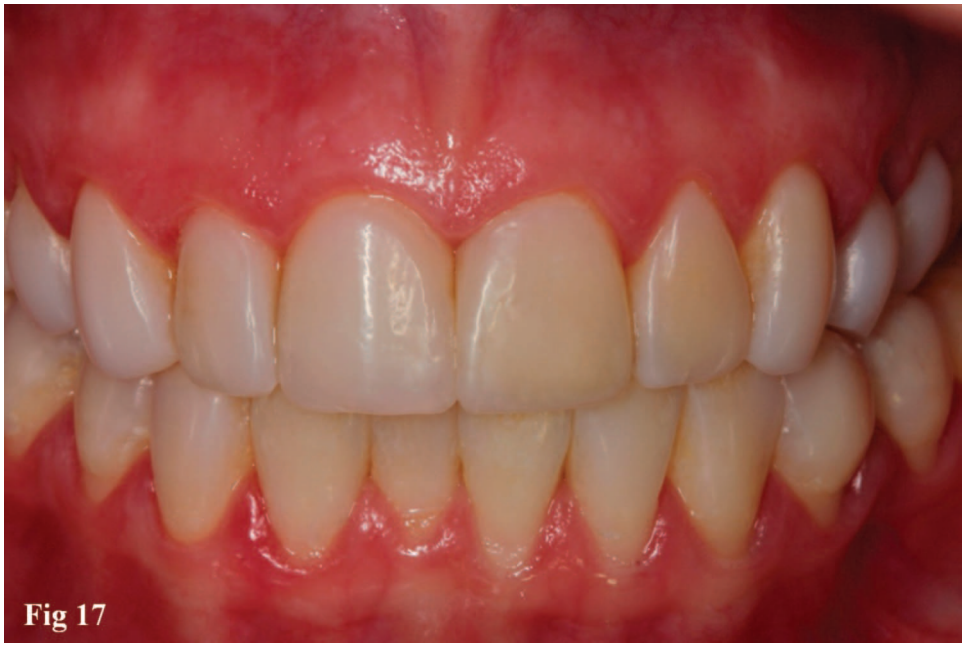


Figure 17. *Case 2: Final clinical result.*

of restorative material.[14] A randomized, split-mouth clinical study reported by Gresnigt and others[15] evaluated the survival rate of direct laminate veneers made of two resin-composite materials. Clinical performance of the two micro-hybrid composite laminate veneers showed a similar survival rate (87.5%). Besides absolute failures, surface roughness and marginal discoloration were the main qualitative deteriorations observed until the final recall.

In case 2, composite resin was used in the anterior and posterior teeth so that orthodontic treatment could be carried out in the future. An advantage to using composite resin was that the sound enamel was preserved and no type of preparation was needed. However, a concern regarding this treatment is related to the adhesive resistance of the hypoplastic enamel. Yaman and others,[16] in an *in vitro* study, observed that self-etching and etch-and-rinse adhesive systems provide reliable bonding to the enamel affected by hypoplastic AI. Another positive aspect is the use of a transparent index to restore the facial surface of the anterior teeth. The index made from polyvinyl siloxane has an excellent reproduction capability, being able to restore contour, shape, and anatomy according to the diagnostic wax-up.

Ceramic has some advantages when compared with composite resin restorations: it is more esthetic, has greater durability and biocompatibility, and has less plaque accumulation.[17,18] However, the vast majority of teeth receiving porcelain laminate veneers should have some enamel removal, usually approximately 0.5 mm. If dentin is exposed, protection is recommended for the period between preparation and cementation in order to prevent postoperative sensitivity and bacterial invasion.[19]

In case 1, ceramic laminate veneers were selected for rehabilitation of all upper teeth. The decision to use ceramic restorations was based on mock-up and patient concern about esthetics and treatment longevity. Several studies[20-23] demonstrated that ceramic laminate veneers have a low clinical failure rate. According to Gresnigt and others,[24] there was no statistically significant difference in survival rates for up to 36 months compared with composite laminate veneers. However, surface quality changes were more frequently observed in composite veneers. In addition, good oral hygiene and absence of parafunctional habits led to the choice of ceramic veneers. A clinical study by Granell-Ruíz and others[25] found that the presence of fractures and debonding of ceramic laminate veneers increased considerably in patients with bruxism. The mock-up indicated no need to extend the preparation depth because the space necessary for the laminate already existed. Based on this, the enamel surface was just regularized to provide a uniform adaptation of the ceramic restoration.

The selection criteria for the two different materials used in rehabilitation of AI patients can be summarized by the following: 1) disorder type and severity, 2) patient age, 3) esthetic demand, 4) treatment longevity, 5) presence or absence of parafunctional habits, 6) oral hygiene, and 7) financial cost. Proper diagnosis and good treatment planning are fundamental to obtaining a satisfactory result for rehabilitation of patients with AI.

CONCLUSION

In both cases presented, the AI disorder type was not very severe. Therefore, less invasive techniques could be performed; case 1 and case 2 could be rehabilitated with ceramic veneers and direct composite resin restorations, respectively. Both treatments have advantages and disadvantages and can be used to successfully restore esthetics and function in patients with AI.

Regulatory Statement

This work was conducted in accordance with all the provisions, guidelines, and policies of the Universidade Federal de Santa Catarina, Florianópolis, Brazil.

Conflict of Interest

The authors 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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Restorative Technique Selection in Class IV Direct Composite Restorations: A Simplified Method

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WW Brackett • MG Brackett

Clinical Relevance

Esthetic resin composite anterior restorations using the so-called multilayer technique may be accomplished when a detailed selection of the shade and an accurate reproduction of the tooth morphology are available.

SUMMARY

Use of the techniques presented here will yield highly esthetic resin composite restorations in minimal time. Although more elaborate composite layering techniques exist and may be used in complex esthetic scenarios, a simplified approach combining two body shades and implementing basic dental anatomy concepts often will deliver highly acceptable esthetic results.

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INTRODUCTION

Reproducing esthetically pleasant anterior restorations requires that clinicians combine artistic skills with fundamental knowledge of tooth morphology, along with selection and use of appropriate composite resin materials.[1] According to Fahl, "This involves comprehensive understanding of tooth shape, color and function and the teeth's natural optical properties in order to select the most appropriate replacement materials."[2]

Today's composite resin systems offer the clinician various enamel and dentin shades to mimic the variations of tooth opacities and translucencies.[3,4] Their main objective is to allow replication of the combined optical properties of dentin and enamel. For small anterior class III or V restorations, only one shade may be necessary, because composite resin is relatively translucent, allowing the adjacent and underlying tooth structure to reflect or show through the restoration.[5] However, for larger through-and-through class III and IV restorations, which have no backing tooth structure, a relatively translucent composite may not be able to mask the dark background of the oral cavity.[6] Therefore, the multilayer technique is recommended, in which an opaque material is placed beneath a translucent

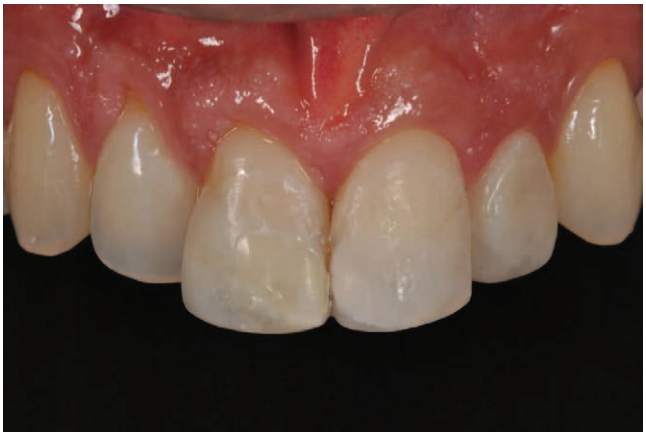


Figure 1. *Inadequate layering technique with compromised results.*

composite resin in an effort to create depth from within the restoration and to mask the dark background.[7] The decision of when to use this technique involves three considerations. According to Vargas,[8] if the adjacent teeth or the tooth to be restored in a through-and-through preparation is polychromatic in nature and no incisal halo or translucency is evident, the tooth may be restored with two shades of composite resin; otherwise, translucent and white opaque shades are indicated to restore the incisal translucency or halo effect. Once the decision is made to use more than one shade, the clinician needs to know the level of translucency of the composite resins being used, because in certain brands, a 2-mm thickness of the body shade (referred to as Universal) of composite resin may be enough to mask the dark background of the oral cavity.[9]

Finally, it is important before restoration to evaluate the tooth morphology (line angles, developmental grooves, and superficial texture) and how to reproduce those details by sculpting the composite and contouring with finishing burs and disks.

The purpose of this article is to describe in detail how one patient's maxillary central incisors were restored using a direct composite resin technique. The previously placed layered class IV resin composite restorations on both central incisors were removed, and the patient's smile was enhanced using a two-shade simplified buildup technique.

CLINICAL CASE

Diagnosis

A caries-free 25-year-old male patient expressed dissatisfaction with the appearance of his smile after recently performed direct composite resin restorations. During the examination, it was determined that the class IV composite resin restorations on both central incisors did not match in color, contour, or texture. A composite veneer was also placed on the left lateral incisor in order to "align the tooth" with the central incisors. All the restorations contained opaque white and translucent resin composite used in an attempt to simulate the natural appearance of dental tissues. The layering technique used was inadequate, and the final result was compromised (Figure 1). After discussion of alternative treatments, the patient decided on a direct bonding procedure because of fewer visits and affordable cost.

Shade Selection

The right lateral incisor was used for shade selection since it had not been restored. A mild color gradient and translucency in the incisal third was found. A decision was made to replace the existing restorations using a two-shade technique based on Vargas's classification on both central incisors, focusing mainly on establishing ideal contours and texture. Shade A2 body was selected for the dentin aspect of the restoration by placing the shade tab in a horizontal position and matching the middle third of the tab to the middle unrestored third of the left central incisor. The facial enamel shade should generally be one shade lighter, so A1 body was selected for the facial aspect of the restoration. It was not considered necessary to use any opaque or dentin-shaded composite resin. Kalore composite resin (GC America, Inc., Alsip, IL, USA) was chosen for this case due to its optical properties.

In order to assess the needed thickness of the lingual layer using the selected body shade to mask the darkness of the oral cavity, two disks 1 and 2 mm thick were fabricated of shade A2 composite resin and then placed on a white background with a black stripe. This allowed the clinician to see that a 2-mm lingual layer thickness was necessary to create the necessary masking effect (Figure 2).

CLINICAL STEPS

Lingual Putty Matrix

A polyvinylsiloxane impression putty material (Reprosil, Dentsply International, York, PA, USA) lingual matrix was fabricated directly in the patient's mouth using the lingual surface of the remaining tooth structure and existing restorations as guides. After local anesthesia was established via infiltration with 2% Lidocaine with 1:100,000 epinephrine (Xylestesin-A 2%, 3M ESPE, St Paul MN, USA), cotton roll isolation was done.

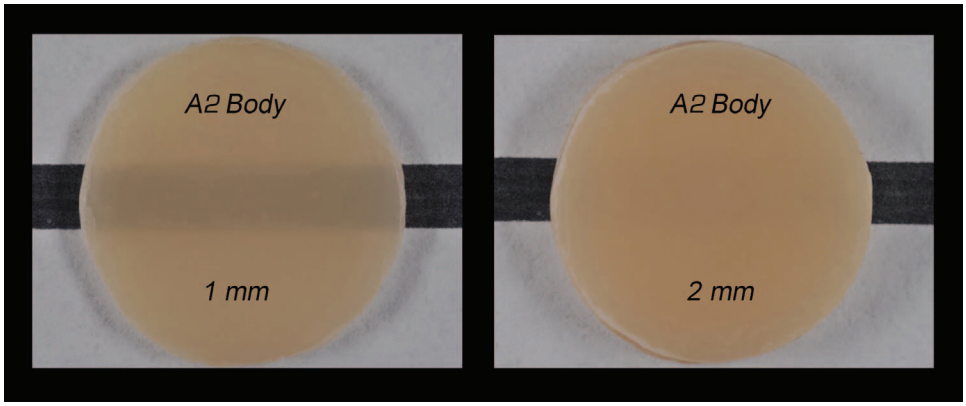


Figure 2. *Masking effect of the selected resin composite product: (Left) 1 mm; (Right) 2 mm.*

Preparation Design

The existing restoration on the right central incisor was removed. A 1.5-mm 75° functional-esthetic enamel bevel was prepared using an 8888 diamond bur (Brasseler, Savannah, GA, USA) on the facial. The lingual bevel was a 45° functional bevel.[10] A coarse disc (Sof-lex, 3M ESPE) was then used to extend the facial bevel interproximally and toward the gingival third of the facial surface to create a so-called "infinite bevel," with which the composite resin margin will be indistinguishable after restoration (Figure 3).[11]

Composite Resin Layering

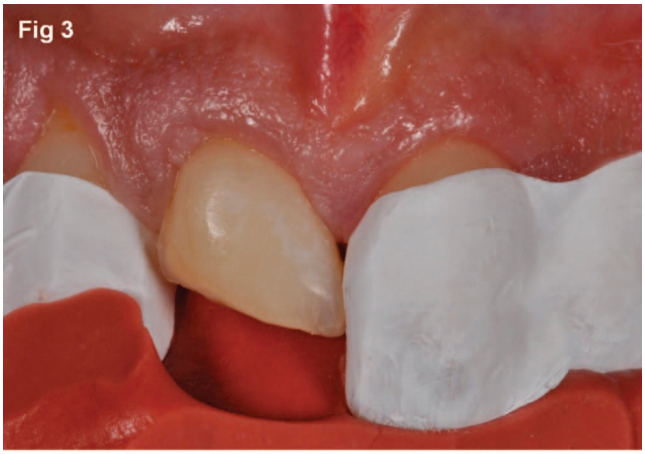
Teflon tape was placed on the adjacent teeth to prevent their being etched. This was followed by the application of 32% phosphoric acid (Uni-Etch, Bisco, Schaumburg, IL, USA) to enamel and dentin for 15 seconds. The acid etchant was then rinsed for 30 seconds, excess water was eliminated, and a dental adhesive (Optibond FL, Kerr, Orange, CA, USA) was applied. This adhesive was considered to provide a more reliable enamel bond than the supplied self-etching adhesive.[12] The lingual PVS matrix was then seated (Figure 3), followed by application of the lingual layer of A2 body shade composite resin to form a lingual shell (Figure 4). After light curing the first increment, the PVS matrix and Teflon tape were removed, and a Mylar strip (Crosstex, Hauppauge, NY, USA) was placed to restore the interproximal walls and contacts. At the same time, thickness was added to the lingual shell (Figure 5). A final 1-mm A1 shade composite resin layer was applied, extending from the facial bevel toward the incisal edge and onto the mesial and distal contact areas to restore the line angles. After polymerization (Valo, Ultradent, South Jordan, UT, USA) of this layer, a thin lead mechanical pencil was used to establish the positions of transitional line angles according to the tooth planes (Figure 6). The main objective was to establish correct lengths and contours (Figure 7). After removal of the composite restoration on the left central incisor (Figure 8), esthetic and functional bevels were prepared, and restoration was completed following the same protocol described above.

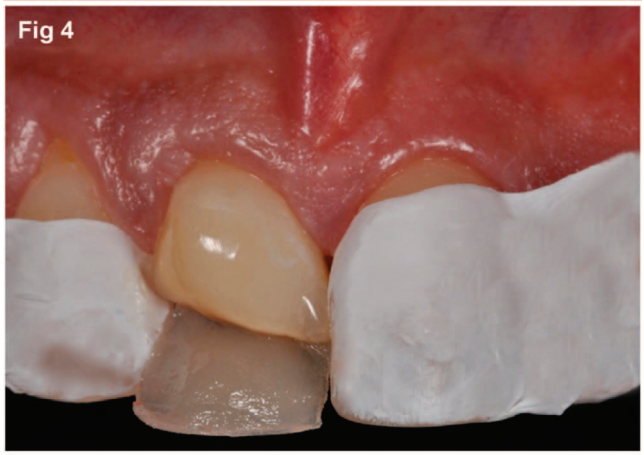
Finishing and Polishing

The finishing process was initiated with coarse and medium-coarse discs (Sof-lex, 3M ESPE) following the contours of the contralateral tooth, followed by the use of the 8888 fine diamond and ET6 extra fine diamond bur (Brasseler) for texture and microanatomy. Finishing strips (Sof-lex, 3M ESPE) were used interproximally to eliminate flash and coarse, and medium and fine rubber polishing points were used on the lingual surface (Jiffy Polishers, Ultradent) after occlusal adjustment (Figure 9). Final esthetic evaluation of shade and texture of the restoration was done 15 days postoperatively (Figure 10).

DISCUSSION

The existing restorations with which the patient presented to the dental office are an example of how lack of planning and understanding of the way that different opacities and translucencies of composite resin behave will compromise restorations.[13] During the shade selection process, the main goal was to select a dentin shade that matched the area of the tooth that is less affected by extrinsic or intrinsic factors.[3] The cervical third of a tooth is affected by the surrounding gingival tissue (extrinsic), which adds red or pink to the existing dental shade. On the other hand, the incisal third of the tooth is affected by the presence of different intrinsic opacities and translucencies, leaving the middle third of the tooth as the area least affected by these factors. Another important factor to consider is the





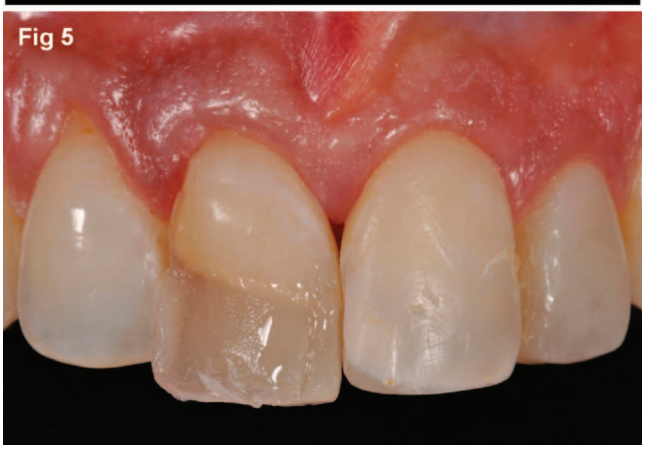
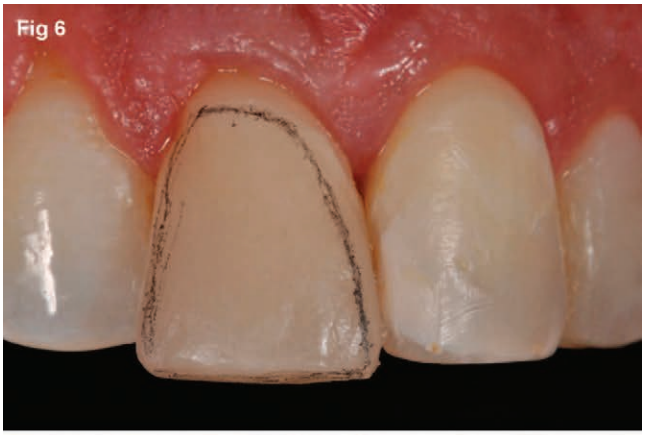
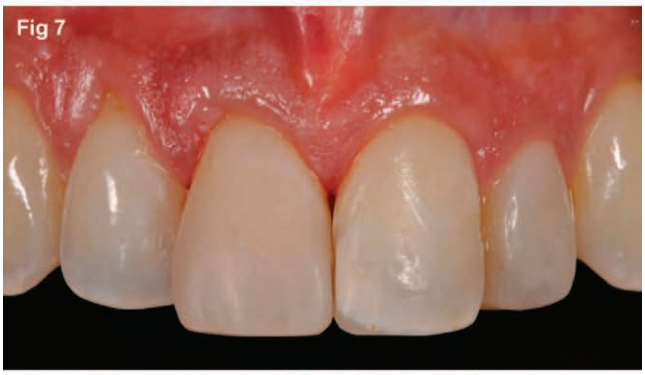


Figure 3. *Facial esthetic (infinite) bevel and PVS matrix and protection of neighboring teeth.*
Figure 4. *A2 lingual shell.*
Figure 5. *Proximal contacts and final lingual thickness established.*

type of shade tab that was used. This was fabricated to reproduce the natural color gradient of teeth, and only the middle third of the tab represents the actual composite shade, so matching these areas will give the dentin shade that, if used for a two-shade technique, will represent approximately 80% of the restoration.[10]





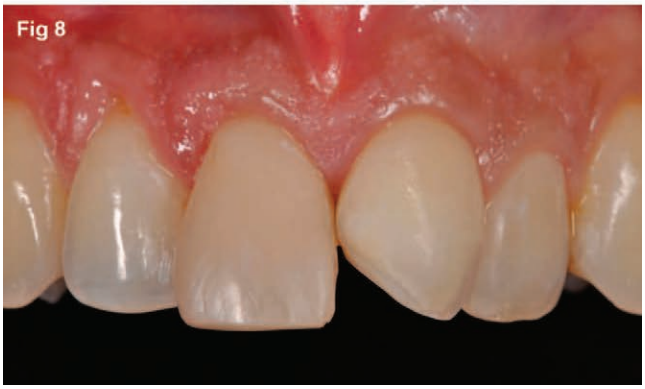
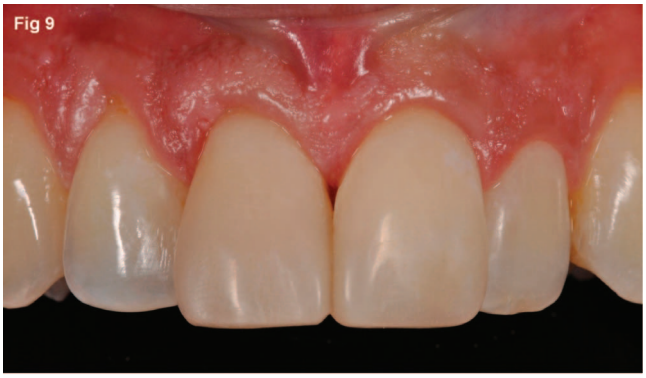


Figure 6. *Facial layer placed and transitional line angles marked.*
Figure 7. *Correct length and contours established on tooth 8.*
Figure 8. *Removal of previous restoration from tooth 9.*

The length and size of the central incisors of this patient when he presented were adequate for making an intraoral putty matrix. Otherwise, it would have been necessary to complete a diagnostic wax-up. Minor adjustments of the existing restorations' lingual contours were performed with a football shape carbide bur (OS1, Brasseler), and lingual embrasures were rectified with a coarse disc (Sof-lex, 3M ESPE) prior to fabricating the lingual matrix. The eventual thickness of the lingual layer of these restorations was approximately 2 mm, which is enough to create the needed opacity to hide the



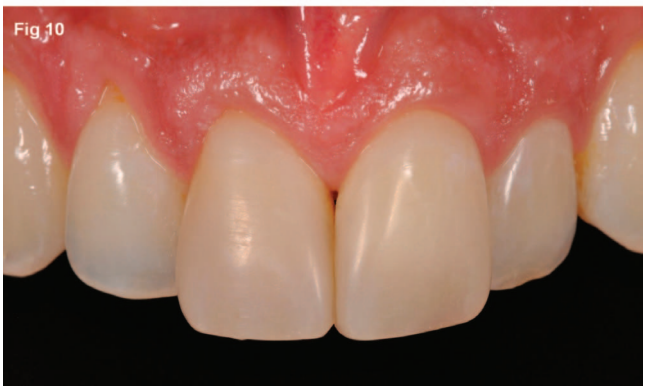


Figure 9. *Final contours and polished surface.*
Figure 10. *Final evaluation done 15 days postoperatively.*

interface of tooth and restoration and to mask any possible shadows. This layer also matched the dentin shade of the underlying tooth structure while leaving space for the final composite layer that replaced the enamel (Figure 5).[9]

Development of natural contours in the final composite layer using three separate increments is recommended.[10] The first and second increments should recreate the mesial and distal line angles. Placement of the composite resin for these should begin at the cervical extension of the esthetic bevel and continue toward the incisal edge against the Mylar strip. This should be followed by slowly pulling the Mylar strip to the lingual. This will result in well-defined line angles prior to light curing.[14] The final increment should be a flatter layer of resin composite filling the area between the line angles, where developmental grooves may be sculpted as needed. The finishing and polishing process can be challenging as we need to recognize when to go from one disk or bur to another. Our recommendation is to follow a five-step sequence. Step 1 can be accomplished by using a coarse Sof-lex disk (3M ESPE) facing down (facing toward the head of the hand piece) or a medium 8888 diamond bur (Brasseler). This should achieve an adequate emergence profile (right central incisor) or blend the resin to tooth interface (left central incisor). Step 2 should establish the correct length using a coarse Sof-lex disc (3M ESPE) facing up (facing away from the head of the hand piece) and incisal embrasures using a medium Sof-lex disc (3M ESPE) facing down. Using discs for this step will give better control of the reduction. Step 3 can be accomplished with medium and fine Sof-lex discs (3M ESPE) facing down and should recreate facial and lingual embrasures. Step 4 should reproduce any secondary anatomy in the incisal third using a medium 8888 diamond bur (Brasseler), whereas step 5 should create a polished surface that resembles the texture present in neighboring teeth by using fine and super fine Sof-lex discs (3M ESPE) facing down.

Although different manufacturers' resin composite and adhesive systems were combined to treat this patient, it has been demonstrated that etch-and-rinse adhesive systems can be safely used with composites from different manufacturers without compromising bond strength.[15] The three-step etch-and-rinse adhesive system was used instead of the self-etching adhesive system supplied by the resin composite manufacturer because it provides a more reliable enamel bond and has been demonstrated in many clinical trials to be very effective.[12,16] In addition, both manufacturers claim that the products used in this case are compatible.

Conflict of Interest

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

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Clinical Research

Two-year Randomized Clinical Trial of Self-etching Adhesives and Selective Enamel Etching

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Clinical Relevance

Selective enamel etching in combination with self-etching adhesives does not affect the overall clinical performance of composite restorations.

SUMMARY

Objective: The aim of this randomized, controlled prospective clinical trial was to evaluate the clinical effectiveness of restoring noncarious cervical lesions with two self-etching adhesive systems applied with or without selective enamel etching.

Methods: A one-step self-etching adhesive (Xeno V$^+$) and a two-step self-etching system (Clearfil SE Bond) were used. The effectiveness of phosphoric acid selective etching of enamel margins was also evaluated. Fifty-six cavities were restored with each adhesive system and divided into two subgroups (n=28; etch and non-etch). All 112 cavities were restored with the nanohybrid composite Esthet.X HD. The clinical effectiveness of restorations was recorded in terms of retention, marginal integrity, marginal staining, caries recurrence, and postoperative sensitivity after 3, 6, 12, 18, and 24 months (modified United States Public Health Service).

Results: The Friedman test detected significant differences only after 18 months for marginal staining in the groups Clearfil SE non-etch (p=0.009) and Xeno V$^+$ etch (p=0.004). One restoration was lost during the trial (Xeno V$^+$ etch; $p>0.05$).

Conclusions: Although an increase in marginal staining was recorded for groups Clearfil SE non-etch and Xeno V$^+$ etch, the clinical effectiveness of restorations was considered acceptable for the single-step and two-step self-etching systems with or without selective enamel etching in this 24-month clinical trial.

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INTRODUCTION

Adhesive systems have gone through several changes in recent years in an attempt to simplify bonding procedures without compromising adhesion to tooth substrates.[1,2] A few years ago, most adhesives were available in three application steps, which were combined into two steps (etch-and-rinse or self-etching), and later, into one single self-etching application step. One-step self-etching adhesives present a shorter clinical application time, reduction in technique sensitivity, and are user-friendly. Despite the simplified approach of all-in-one adhesives, early formulations did not promote an effective seal of dentin.[1] However, manufacturers claim that the chemistry behind newer all-in-one self-etching adhesives have been changed for improved performance.[3,4]

Self-etching systems have been widely accepted as a good alternative for bonding resin composite to dentin. However, controversy still remains regarding their use for bonding composite to enamel.[5,6] This concern centers around the shallower demineralization pattern produced by mild self-etching systems compared with etch-and-rinse systems.[7,8] Therefore, selective enamel etching with phosphoric acid has been routinely indicated when self-etching systems are to be used. A long-term clinical study has demonstrated only minor benefits of selective etching of enamel margins when a two-step self-etching system was used.[9] However, there is no information on whether selective etching is necessary for newer single-step self-etching formulations.

Laboratory-based studies are important for predicting the clinical performance of adhesive procedures, whereas randomized clinical trials are the ultimate tests to evaluate the clinical efficacy of adhesive materials and techniques.[10-12] Noncarious cervical lesions (NCCLs) are widely available and are normally used because they present no macromechanical retention, they present margins in enamel and dentin, and are subjected to high stress during masticatory function.[13,14]

The null hypotheses of this randomized, controlled prospective clinical trial were that 1) there is no difference in the long-term clinical performance of NCCLs restored with a two-step and a one-step self-etching system; and 2) selective etching of enamel margins produces no difference in the long-term clinical performance of restorations.

METHODS AND MATERIALS

Two self-etching adhesives were evaluated in the present investigation: a one-step, XENO V$^+$ (Dentsply De Trey, Konstanz, Germany), and a two-step, Clearfil SE Bond (Kuraray Noritake, Tokyo, Japan). Clinical effectiveness of adhesive systems was evaluated when they were applied following the manufacturers' recommendations, abbreviated as XV-NE (non-etch) and CSE-NE, and when applied after selective etching of enamel margins with 36% H_3PO_4, abbreviated as XV-E (etch) and CSE-E. Composition, manufacturers, and application technique of materials are presented in Table 1.

Clinical effectiveness of restoration was determined according to the following parameters: retention rate, marginal integrity, marginal staining, secondary caries, postoperative sensitivity, and pulp vitality. Clinical performance of restorations was evaluated at baseline and at 3, 6, 12, 18, and 24 months of clinical service. Clinical success was recorded according to the modified United States Public Health Service (USPHS) criteria.[10]

Fifty-six class V restorations were performed with each adhesive and were divided into two subgroups (n=28; with or without selective enamel etching).

Inclusion and Exclusion Criteria

Previous to patient recruitment, the research protocol was approved by the Ethics Committee in Clinical Research. This clinical trial was registered at ClinicalTrials.gov. Patients were examined by a single investigator and needed at least four NCCLs, independent of tooth location. Patients with a compromised medical history, severe or chronic periodontitis, extreme caries sensitivity, heavy bruxism, under orthodontic treatment, having poor oral hygiene and smokers were excluded from the study. Based on these criteria, 25 patients were included in the present investigation and signed the informed consent.

Prior to restoration, lesions were classified in terms of shape, depth, cervico-incisal size, degree of dentin sclerosis, presence of antagonist, preoperative sensitivity, and type of tooth.

Restorative Procedure

Operative procedures were performed by an experienced dentist from the Department of Operative Dentistry. Each patient received at least four restorations, in which groups were randomly allocated (using randomization tables). Four restorations were placed in one appointment. Three patients had eight lesions, which were restored in two appointments. After shade selection, teeth were restored using cotton roll and retraction cord (Ultra-

Table 1: *Materials, Manufacturers, Lot Number, Composition, and Application Technique*

Materials	Composition	Application procedure
Clearfil SE (Kuraray-Noritake) Lot# Primer 00954A; Bond 01416A	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, CQ, *N,N*-diethanol p-toludine, water	Apply primer for 20 seconds; gently air-blow
	Bond: 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, CQ, *N,N*-diethanol p-toludine, silanized colloidal silica	Apply adhesive and light-cure for 10 seconds
Xeno V^+ (Dentsply DeTrey) Lot# 00751	Bifunctional acrylate, acidic acrylate, functionalized phosphoric acid ester, water, tertiary butanol, initiator, stabilizer	Apply adhesive for 20 seconds, gently air-blow and light-cure for 10 seconds
DeTrey Conditioner 36 (Dentsply DeTrey) Lot# 1004002386	Phosphoric acid, highly dispersed silicon dioxide, detergent, pigment, water	Apply etchant selectively on enamel and leave for 15 seconds; thoroughly rinse and gently air dry (only for CSE-E and XV^+-E)
Esthet.X HD (Dentsply Caulk) Lot# 100726	Bis-GMA, Bis-EMA, triethylene glycol dimethacrylate, CQ, Stabilizer, pigments, barium fluoroborosilicate glass, nanofiller silica	Apply increments (maximum thickness of 2 mm) and light cure for 20 seconds

Abbreviations: Bis-GMA, bisphenol-A glycidyl dimethacrylate; Bis-EMA, bisphenol-A ethoxylated dimethacrylate; CQ, di-camphorquinone; HEMA, hydroxyethyl methacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate.

pack #000 or 00, Ultradent, Salt Lake City, UT, USA) isolation. Lesions were cleaned with pumice and water in a rubber cup followed by rinsing and drying. An enamel bevel of 1 to 2 mm was prepared with a fine diamond bur (#1190F, FG 314 ISO no. 890, 010, grit size 45 μm, KG Sorensen, Cotia, São Paulo, Brazil) operated in a high-speed handpiece under air-water spray.

For groups with selective enamel etching, margins were etched with 36% H_3PO_4 (De Trey Conditioner, Dentsply De Trey, Konstanz, Germany) for 15 seconds and subsequently thoroughly rinsed and air-dried. Adhesive systems were applied according to the manufacturers' instructions and light-cured with a light emitting diode (LED) having a power output of 1500 mW/cm^2 for 10 seconds (Radii Plus, SDI, Bayswater, Australia). NCCLs were restored incrementally with a microhybrid composite resin (Esthet.X HD, Dentsply Caulk, Milford, DE, USA). Increments were light cured for 20 seconds. Afterward, the retraction cord was removed, and finishing/polishing was performed with rubber points under water spray (Enhance/PoGo, Dentsply Caulk).

Evaluation Criteria

Restorations were evaluated at baseline and 3, 6, 12, 18, and 24 months of clinical service for retention, marginal integrity, marginal staining, postoperative sensitivity, caries recurrence, and pulp vitality according to the modified USPHS criteria.[10,15] High-resolution photographs were made preoperatively, at baseline, and at each recall (DSLR Camera EOS Rebel T4i, Macro lens EF 100 mm, Flash Twin Lite MT-24EX, Canon Inc, Tokyo, Japan). Two independent examiners blinded to the adhesive systems and technique carried out all evaluations. Any discrepancy between examiners was resolved at chair side.

The statistical analyses followed the intention-to-treat protocol according to the Consolidated Standards of Reporting Trials.[16] This protocol includes all participants in their originally randomized groups, even those who were not able to keep their scheduled recall visits. This approach is more conservative and less open to bias.[2] The Friedman test was used for statistical analysis of retention rate, marginal integrity, marginal pigmentation, caries recurrence, postoperative sensitivity, and pulp vitality at the 5% confidence level.

RESULTS

A description of NCCL classification and distribution is presented in Table 2. The majority of lesions (61.6%) presented a cervico-incisal height >2.5 mm. Most of the cavities presented some degree of dentin sclerosis (83.9%). In addition, patients reported preoperative sensitivity in 52.7% of lesions. The clinical data for the different parameters evaluated at different time intervals are presented in Table 3. The recall rate at 3 and 6 months was 100%. At the 12- and 18-month evaluation periods, the recall rate was 96.4% (one patient having one restoration allocated in each group had all teeth extracted for implant placement). At the 24-month evaluation, the recall rate dropped to 92.9% (one patient moved to another city and through telephone contact related that no restoration was lost).

Table 2: *Distribution of Noncarious Class V Lesions According to Patient Sex; Shape, Depth, and Cervico-Incisal Size of the Lesion; Degree of Sclerotic Dentin; Presence of Antagonist; Presence of Preoperative Sensitivity; and Type of Tooth*

Characteristic of class V lesions	Number of lesions	%
Total	112	100
Patient sex		
Male 13	60	53.5
Female 12	52	46.5
Shape and depth		
Wedge-sharp, ≤1 mm depth	34	30.4
Wedge-sharp, >1 mm depth	36	32.1
Saucer-rounded, ≤1 mm depth	33	29.5
Saucer-rounded, >1 mm depth	9	8
Cervico-incisal height		
<1.5 mm	5	4.5
1.5–2.5 mm	38	33.9
>2.5 mm	69	61.6
Degree of sclerotic dentin		
No sclerosis	18	16.1
Slight sclerotic dentin (opaque)	47	42
Moderate sclerotic dentin (yellow)	24	21.4
Severe sclerotic dentin (transparent)	23	20.5
Presence of antagonist		
Antagonist present	105	93.8
Antagonist not present	7	6.3
Pre-operative sensitivity (to air and/or tactile contact)		
Yes	59	52.7
No	53	47.3
Tooth distribution		
Lower incisor	2	1.8
Lower canine	3	2.7
Lower premolar	29	25.9
Lower first molar	2	1.8
Upper incisor	13	11.6
Upper canine	11	9.8
Upper premolar	47	42
Upper first molar	5	4.5

For retention rate, no significant differences were observed among groups ($p>0.05$). One restoration was lost, from a patient in group XV-E at the 12-month recall. No significant differences were observed for marginal integrity among groups ($p>0.05$). However, a few minor superficial marginal defects were observed on enamel margins for group CSE-NE (3.6%) at the 12-, 18-, and 24-month recalls; for group XV-E (3.7%) at the 18- and 24-month recalls; and for group XV-NE (3.6%) at the 18- and 24-month recalls. Group CSE-E did not present any marginal defects throughout the study.

A significant increase in marginal discoloration was observed after 18 months of clinical service for groups CSE-NE ($p=0.009$) and XV-E ($p=0.004$). Small areas of discoloration were observed on enamel margins for XV-E, which increased with time (3.6% at 6-month, 7.4% at 12-month, 11.1% at 18-month, and 14.8% at 24-month recalls). The same trend was observed for CSE-NE (3.6% at 12-month, 10.7% at 18-month, and 14.3% at 24-month recalls) as shown in Figure 1. No significant differences were detected in marginal discoloration for CSE-E ($p>0.05$). Also, no significant difference was detected for XV-NE ($p>0.05$), although a trend toward increased marginal discoloration was observed (7.1% at 24-month recall).

For postoperative sensitivity, 100% of patients reported no sensitivity in any recall period ($p>0.05$). Secondary caries were not observed in any group ($p>0.05$). The overall clinical success was not significantly different among groups ($p>0.05$). Loss of one restoration was recorded for group XV-E at the 12-month recall (96.4% overall clinical success); for the other groups, overall clinical success was 100%.

DISCUSSION

Despite being considered user-friendly, single-step adhesive systems were often criticized due to low clinical performance. Acidity (pH) adjustment of adhesive solution and incorporation of new functional monomers to promote clinical performance stability over time were the main changes proposed to improve these materials. In this study, Clearfil SE Bond (CSE) was chosen as the control, because it is considered the gold standard for self-etching adhesives and demonstrates a clinical performance similar to the three-step etch-and-rinse.[9,17,18]

CSE acidic primer contains 10-methacryloyloxydecyl-dihydrogen-phosphate (10-MDP) dissolved in water, with a pH of around 2. This promotes a mild dentin surface etching, resulting in a thin but uniform and stable hybrid layer.[19] In addition, an interaction occurs between 10-MDP and hydroxyapatite crystals present around and within collagen fibrils of the hybrid layer.[18,20] Results of this study corroborate data obtained by Peumans and others,[18] who also evaluated CSE for 13 years, with the same

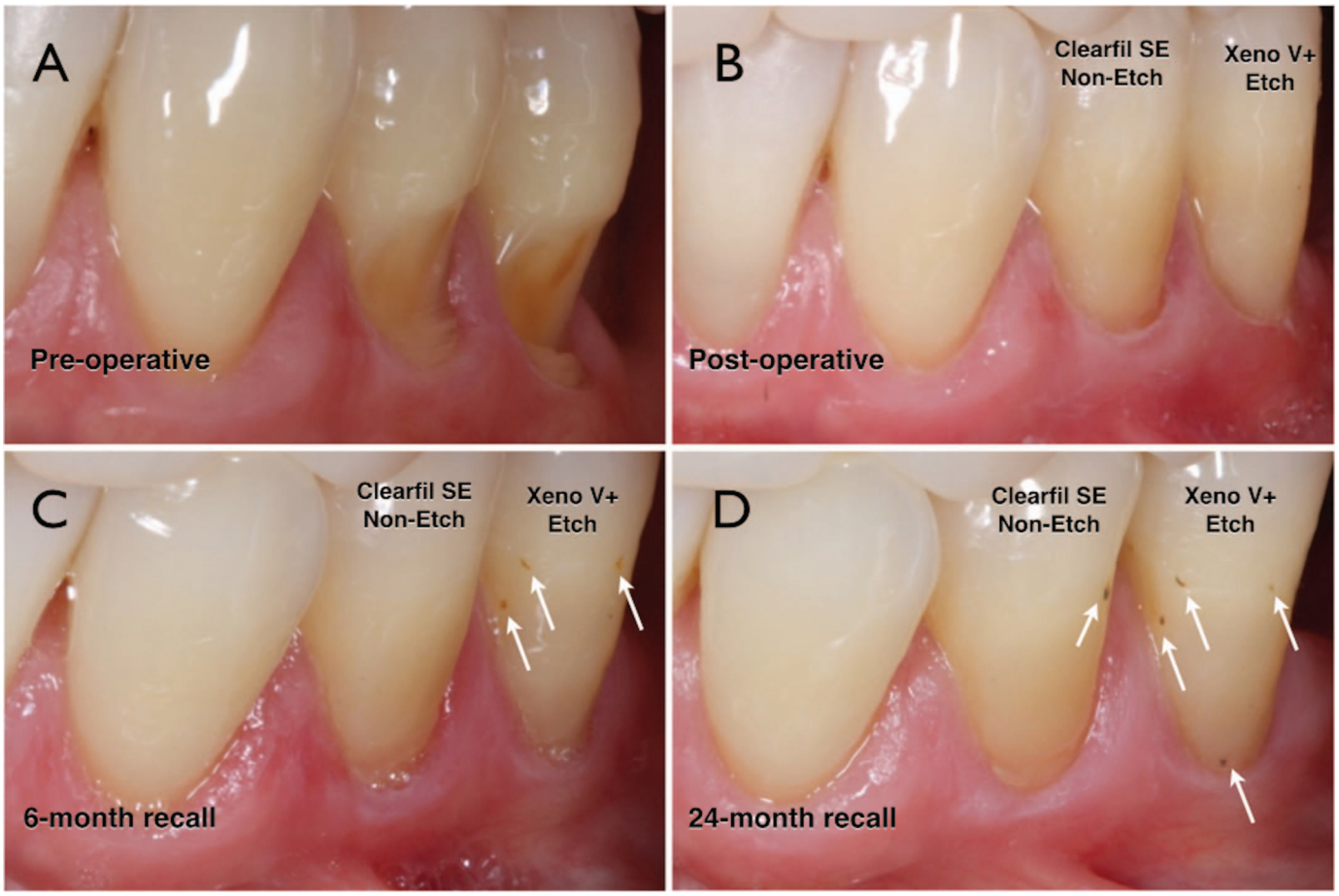

Figure 1. *(A) Preoperative view of noncarious cervical lesions. (B) Aspect immediately after placement of restoration. (C) After 6 months, regions of marginal discoloration were observed for XENO V+ E. (D) After 24 months, marginal discoloration was also observed for CSE-NE. Arrows point to regions of marginal discoloration.*

enamel treatment. Selective enamel etching produced only minor positive effects on NCCLs.[18] Another factor that contributes to satisfactory performance of CSE is the application of a separate hydrophobic-filled adhesive layer. This layer may also contribute to withstand stresses generated in the composite/dentin interface during polymerization.[21,22]

Van Meerbeek and others[11] showed a 100% retention rate for CSE after 24 months, with marginal staining in enamel being the only difference found in the group that did not receive selective etching after 24 months (there was no assessment at 18 months). However, according to the authors, this finding did not determine the need for restoration replacement. Similar findings were obtained in this study; however, a significant increase was observed for marginal staining after 18 and 24 months. The highest marginal enamel staining for CSE-NE is probably due to the moderate CSE pH, which was not sufficient to produce an adequate etching pattern compared with phosphoric acid.[11,23]

XENO V^+ is an evolution of the adhesive family XENO III (Dentsply DeTrey), XENO IV (Dentsply Caulk), and XENO V (Dentsply DeTrey). This latest version (XENO V^+) contains a phosphonated pentacrylate ester (PENTA)-modified monomer, reduced the curing time and the aroma to a softened butyl alcohol odor. In the present study, there was no statistical difference in most of the parameters studied. Restorations performed with XENO V^+ showed promising results in NCCL restorations after 2 years of clinical follow-up. XENO V^+ is a recent material with few clinical and laboratory tests available comparing it with other adhesives.[24,25] Therefore, comparison with the gold standard self-etching adhesive (CSE) in a 2-year clinical trial produces relevant clinical data. XENO V^+ presents a

Table 3: *Results of Evaluated Parameters in Percentage at Each Recall Period*

Evaluated parameters	Recall period											
	3 months				6 months				12 months			
	CSE		XenoV+		CSE		XenoV+		CSE		XenoV+	
	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE
Recall rate	100				100				96.4			
Retention rate	100	100	100	100	100	100	100	100	100	100	96.4	100
	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(27)	(28)
Absence of marginal defects	100	100	100	100	100	100	100	100	100	96.4	100	100
	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(27)	(27)	(28)
Enamel marginal defect	0	0	0	0	0	0	0	0	0	3.6 (1)	0	0
Small enamel marginal defect	0	0	0	0	0	0	0	0	0	3.6 (1)	0	0
Severe enamel marginal defect	0	0	0	0	0	0	0	0	0	0	0	0
Dentin marginal defect	0	0	0	0	0	0	0	0	0	0	0	0
Small dentin marginal defect	0	0	0	0	0	0	0	0	0	0	0	0
Severe dentin marginal defect	0	0	0	0	0	0	0	0	0	0	0	0
Absence of marginal discoloration	100	100	100	100	100	100	96.4	100	96.4	96.4	92.6	100
	(28)	(28)	(28)	(28)	(28)	(28)	(27)	(28)	(27)	(27)	(25)	(28)
Superficial localized marginal discoloration	0	0	0	0	0	0	3.6 (1)	0	3.6 (1)	3.6 (1)	7.4 (2)	0
Deep generalized marginal discoloration	0	0	0	0	0	0	0	0	0	0	0	0
Absence of sensitivity	100	100	100	100	100	100	100	100	100	100	100	100
	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(27)	(28)
Absence of caries occurrence	100	100	100	100	100	100	100	100	100	100	100	100
	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)
Overall clinical success rate	100	100	100	100	100	100	100	100	100	100	96.4	100
	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(27)	(28)

Number of noncarious cervical lesions is presented between parentheses.
[a] *Underlined values are significantly different by Friedman test at the 95% confidence level.*

pH of approximately 1.3, being considered more aggressive compared with SE Bond. Thus, the indication of selective etching with this material must be questioned.

Groups CSE-E, CSE-NE, and XV-NE presented a 100% retention rate after 24 months, whereas the XV-E group had a retention rate of 96.4%, due to the loss of one restoration at the 12-month evaluation recall. This small difference after 2 years did not result in statistically significant differences between materials ($p>0.05$). Results obtained by XENO V^+ may be due to the change of the ester group by an amide group that provided these monomers higher stability in acidic and aqueous medium.[25] It has been suggested that hydrogen bonds can occur between amide-based monomers and collagen carboxyl groups.[26,27]

Relative to marginal integrity, CSE-E presented 100% of intact margins, whereas CSE-NE, XV-E, and XV-NE presented approximately 96%. Marginal problems detected were only small surface enamel defects (24 months), requiring finishing and repolishing of restorations. There was no statistical difference between groups. Similar findings were observed by Van Meerbeek and others,[11] in which CSE was the subject of study.

For marginal staining, CSE-E (96.4%) and XV-NE (92.9%) presented no statistically significant difference; however, they differed significantly from CSE-NE (85.7%) and XV-E (85.2%), which were similar after 24 months. Enamel staining was small and superficial, without the need for restoration replacement. Despite some improvement with regard to marginal staining for CSE, selective enamel etching

Table 3: *Results of Evaluated Parameters in Percentage at Each Recall Period (ext.)*

Evaluated parameters	Recall period							
	18 months				24 months			
	CSE		XenoV+		CSE		XenoV+	
	E	NE	E	NE	E	NE	E	NE
Recall rate	96.4				92.9			
Retention rate	100	100	96.4	100	100	100	96.4	100
	(28)	(28)	(27)	(28)	(28)	(28)	(27)	(28)
Absence of marginal defects	100	96.4	96.3	96.4	100	96.4	96.3	96.4
	(28)	(27)	(26)	(27)	(28)	(27)	(26)	(27)
Enamel marginal defect	0	3.6 (1)	3.7 (1)	3.6(1)	0	3.6(1)	3.7(1)	3.6(1)
Small enamel marginal defect	0	3.6 (1)	3.7 (1)	3.6(1)	0	3.6(1)	3.7(1)	3.6(1)
Severe enamel marginal defect	0	0	0	0	0	0	0	0
Dentin marginal defect	0	0	0	0	0	0	0	0
Small dentin marginal defect	0	0	0	0	0	0	0	0
Severe dentin marginal defect	0	0	0	0	0	0	0	0
Absence of marginal discoloration	96.4	89.3	88.9	96.4	96.4	85.7	85.2	92.9
	(27)	(25)	(24)	(27)	(27)	(24)	(23)	(26)
Superficial localized marginal discoloration	3.6	10.7	11.1	3.6	3.6	14.3	14.8	7.1
	(1)	(3)	(3)	(1)	(1)	(4)	(4)	(2)
Deep generalized marginal discoloration	0	0	0	0	0	0	0	0
Absence of sensitivity	100	100	100	100	100	100	100	100
	(28)	(28)	(27)	(28)	(28)	(28)	(27)	(28)
Absence of caries occurrence	100	100	100	100	100	100	100	100
	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)
Overall clinical success rate	100	100	96.4	100	100	100	96.4	100
	(28)	(28)	(27)	(28)	(28)	(28)	(27)	(28)

did not promote additional benefits for XENO V^+ after 24 months. Differences in marginal staining between CSE-E and CSE-NE have been attributed to a shallower enamel-etching pattern obtained due to the mild self-etching primer pH.[11,28] The highest marginal staining observed in enamel margins for the XV-E group may be due to exacerbated enamel etching with phosphoric acid combined with the acidic adhesive solution (pH $\sim$ 1.3). A variation in etching time and acidity produces a series of phenomena, weakening enamel structure and not providing an effective etching pattern.[29]

Patients reported preoperative sensitivity in 52.7% of lesions. However, after placement of restorations, none of the groups presented postoperative sensitivity at any recall. It can be speculated that CSE and XENO V^+ provided good sealing of dentinal tubules, confirming that self-etching adhesives perform well in preventing postoperative sensitivity.[9,11] However, as most of the NCCLs presented some level of dentin sclerosis (83.9%), which could cause many of the dentin tubules to be occluded at the start of the study, the insulating effect of the composite resin can also account for the reduction in sensitivity.

Although the degree of dentin sclerosis has been reported to affect restorations in NCCLs,[30] our results corroborate with several studies that found no correlation between longevity of restorations and dentin sclerosis.[9,11,12,31] Also, no correlation has been found between performance of NCCL restorations and patient age, tooth type, size and shape of lesion, location, and occlusal load.[9,11,12,31] Both adhesive systems, with and without enamel selective etching, reached the American Dental Association (ADA) parameters for clinical use (less than 10% of

restoration loss or deep marginal staining in 18 months). Other parameters, such as secondary caries and pulp vitality, were considered excellent in all groups at all evaluation times.

CONCLUSION

Overall clinical success of the two self-etching adhesive systems tested in this study was not affected by selective enamel etching in the 24-month evaluation. There was no significant difference between groups tested for retention rate, marginal integrity, secondary caries, and postoperative sensitivity.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: Guarulhos University Ethics Committee. The approval code for this study is: #636.726.

Conflict of Interest

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

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In Vivo and *In Vitro* Effects of Chlorhexidine Pretreatment on Immediate and Aged Dentin Bond Strengths

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Clinical Relevance

For the tested etch-and-rinse and self-etch adhesives, 2%chlorhexidine application can counteract time-dependent decline in adhesive bonds to dentin, and increase the bonding effectiveness over time.

SUMMARY

This study evaluated the effect of 2% chlorhexidine (CHX) pretreatment of dentin on the immediate and aged microtensile bond strength (μTBS) of different adhesives to dentin *in vivo* and *in vitro*. Class I cavities were prepared in 80 caries-free human third molars of 40 patients in a split-mouth fashion. In each tooth pair, one tooth received 2% CHX pretreatment after which both teeth were randomly assigned to one of the following groups with respect to the type of adhesive system applied: Adper Single Bond 2 (etch-and-rinse), Clearfil SE Bond (two-step self-etch), Clearfil S^3 Bond (one-step self-etch), and Adper Prompt-L-Pop (all-in-one self-etch). The teeth were restored with resin composite and extracted for μTBS testing either immediately or after six months in function. *In vitro* specimen pairs were prepared as with the clinical protocol in intact, freshly extracted human molars, and thereafter, subjected to testing immediately or after 5000× thermocycling. Data were analyzed with four-way analysis of variance (ANOVA). Bonferroni test was utilized for pair-wise comparisons. The immediate bond strength values were significantly higher than "aged" ones for all tested adhesives (p=0.00). The *in vitro* immediate bond strength values were statistically higher than *in vivo* bond strength values (p<0.05). While the bond strength of *in vitro* aged, CHX-treated samples were higher than their *in vivo* counterparts (p<0.05), no difference was observed in non-CHX treated groups (p>0.05). In the

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absence of CHX pretreatment, all adhesives showed significantly higher immediate bond strength values than CHX-treated groups, while all "aged", non-pretreated adhesives exhibited significantly lower bond strength values (both $p<0.05$). By contrast, chlorhexidine pretreatment resulted in significantly higher aged bond strengths, regardless of the adhesive system and testing condition. Aging-associated decline in dentin bond strength of etch-and rinse and self-etch adhesives can be counteracted by chlorhexidine application.

INTRODUCTION

Resin-dentin bonds are less durable than resin-enamel bonds, which might be one reason for the relatively short lifespan of tooth-colored fillings.[1] Degradation of the resin-dentin bond has been associated with water/oral fluid sorption, polymer swelling, and resin leaching *in vitro* and *in vivo*.[2,3] Endogenous matrix metalloproteinases (MMPs) also appear to be involved in the disintegration of hybrid layers over time.[2,4-6] Collagen fibrils exposed during the acid etching procedure become susceptible to hydrolytic and enzymatic degradation, a process mediated by activation of dentin MMPs.[4] Likewise, etch-and-rinse adhesives and self-etching adhesives can activate endogenous MMPs during dentin bonding.[7-9]

Chlorhexidine (CHX) is a nonspecific protease inhibitor[10] that can suppress collagenolytic/gelatinolytic activity of dentin matrices.[4] As CHX has cationic properties, it can bind electrostatically to negative carboxyl groups and hydroxyl groups of collagen and noncollagenous phosphoproteins in demineralized dentin, as well as electrostatically to phosphate groups in hydroxyapatite crystallites in mineralized dentin. Application of CHX at different concentrations has been shown to maintain the hybrid layer preservation in several *in vivo*[11-13] and *in vitro* studies.[14-16] Based on published data, there appears to be an increasing tendency among clinicians to apply CHX on dentin prior to bonding procedures. It has been recommended to apply CHX on etched dentin in association with etch-and-rinse adhesives, before primer and bonding application.[17] The binding of CHX to demineralized dentin is much higher than that to mineralized dentin.[18] There are many results of *in vitro* studies about CHX application before bonding procedure,[14-16,19-22] but these results are yet to be verified *in vivo*. However, the effect of CHX on self-etching adhesives has not been well determined.

Based on these observations, the aim of this clinical and laboratory study was to test the efficacy of 2% CHX pretreatment on the microtensile bond strength (μTBS) of different adhesives to dentin immediately and after aging. The null hypotheses tested were that: 1) CHX pretreatment has no influence on μTBS values to dentin, 2) bond strength is not influenced by *in vivo* or *in vitro* testing, and 3) bond strength values are not related to the tested adhesive systems.

METHODS AND MATERIALS

This study was conducted in both laboratory and clinical phases. The *in vitro* and clinical research protocols and consent forms were evaluated and approved by the Institutional Human Subject Review Committee.

In Vitro Procedures

Eighty freshly extracted intact human third molars were stored in distilled water for up to 30 days. Standardized Class I cavity preparations with 3-mm mesial-distal width, 2-mm buccolingual width, and 2-mm depth were completed using a diamond cylinder bur (Diatech, Swiss Dental Instruments, Heerbrugg, Switzerland) in a water-cooled high-speed handpiece.

The teeth were randomly divided into four groups (n=20) with respect to the type of adhesive system tested: group I, two-step etch-and-rinse (Adper Single Bond 2, 3M ESPE, St Paul, MN, USA); group II, two-step self-etch (Clearfil SE Bond, Kuraray, Tokyo, Japan); group III, one-step self-etch (Clearfil S^3 Bond, Kuraray); and group IV, all-in-one self-etch, (Adper Prompt-L-Pop, 3M ESPE). Prior to adhesive application, specimens in each group were further randomly allocated into two subgroups (n=10): (A) no CHX digluconate pretreatment and (B) 2% CHX digluconate pretreatment. In subgroups B, 2% CHX was applied for 30 seconds using a foam pellet saturated with the solution. Excess CHX was blot-dried prior to application of the adhesive. In group I, CHX was applied after the acid-etching procedure, while in groups II, III, and IV, the cavities were treated with CHX prior to the application of the tested self-etch adhesives. In all groups, the cavities were restored with a hybrid resin composite (Filtek Z250, 3M ESPE). The composite was placed in 2-mm thick increments, each light cured with a LED unit for 20 seconds. The main components, modes of application, and batch numbers of the materials used in the present study are shown in Table 1.

Table 1. Materials, Manufacturers, Batch Numbers, Chemical Composition, and Application Modes

Materials (Batch Number)	Composition	Mode of Application
Adper Single Bond 2 (3M ESPE, MN, USA) (51202)	Etchant: Scotchbond acid-35% phosphoric acid	Apply Scotchbond etchant to enamel and dentin. Wait 15 s. Rinse for 10 s. Blot excess water leaving tooth moist.
	Bis-GMA, HEMA, co-polymer of acrylic/ itaconic acids, diurethane dimethacrylate, glyceroldimethacrylate, water, and ethanol	Adhesive: Using a fully saturated brush tip for each coat, apply two consecutive coats of Adper Single Bond adhesive to etched enamel and dentin. Dry gently for 2-5 s. Light cure for 10 s.
Clearfil SE Bond (Kuraray Medical Inc, Okayama, Japan) (00972A - Primer) (01443A - Bond)	Primer: MDP, HEMA, dimethacrylate monomer, water, catalyst	Prime for 20 s (no mixing required)
	Bond: MDP, HEMA, dimethacrylate monomer, microfiller, catalyst	Apply Bond and light-cure for 10 s.
Clearfil S^3 (Kuraray Medical Inc, Okayama, Japan) (00143A)	MDP, Bis-GMA, HEMA, dl-camphoroquinone, ethanol, water, colloidal silica	Apply bond and wait 20 s. Dry with high-pressure air for 5 s. Light-cure for 10 s.
Adper Prompt-L-Pop (3M ESPE, MN, USA) (387690)	Methacrylated phosphoric esters, Bis-GMA, camphoroquinone, stabilizers, water, 2- (HEMA), polyalkenoic acid	Mix adhesive according to instructions. Apply adhesive with a rubbing motion for 15 s. Gently but thoroughly air-dry to remove the aqueous solvent. Light-cure for 10 s. Apply a second coat. Gently but thoroughly air-dry to remove the aqueous solvent. Light-cure for 10 s.
Klorhex (Drogsan Medicine, Ankara, Turkey) (2006/66)	2% Chlorhexidine digluconate	Use a foam pellet saturated with the solution for 30 s. Blot-dry excess CHX.
Filtek Z250 (3M ESPE, MN, USA) (N110475)	Bis-GMA, UDMA, Bis-EMA resin, zirconium, silica	Insert incrementally in 2-mm increments. Light-cure for 40 s.

Abbreviations: BisEMA, ethoxylated bisphenol-A-glycidyl methacrylate; Bis-GMA, bis-phenol A diglycidylmethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

Restored teeth were stored in distilled water for 24 hours, after which finishing and polishing procedures were accomplished with diamond burs (Diatech) and flexible disks (Sof-Lex Pop-on, 3M ESPE), respectively. In each subgroup, half of the specimens (n=5) were randomly assigned for immediate bond strength testing (ie, after 24 hours), while the other half was subjected to thermocycling (5000X) in a water bath from 5°C to 55°C with a dwell time of 30 seconds at each temperature and a transfer time of 5 seconds. All specimens were stored in distilled water until bond strength testing.

In Vivo Procedures

Forty 23- to 28-year-old healthy volunteers with a pair of occluding, noncarious, contralateral third molars scheduled for future extraction were enrolled for *in vivo* sample preparation. Class I cavities conforming to the dimensions of the *in vitro* counterparts (3 mm mesial-distal, 2 mm buccolingual, 2 mm deep) were prepared in third molars under rubber dam isolation. As with the *in vitro* part, patients were randomly allocated into four groups with respect to the adhesive system applied: group V, two-step etch-and-rinse (Adper Single Bond 2); group VI, two-step self-etch (Clearfil SE Bond); group VII, one-step self-etch (Clearfil S^3 Bond); and group VIII, all-in-one self-etch, (Adper Prompt-L-Pop). For each tooth pair, one tooth received adhesive placement without prior CHX application, while the contralateral molar was pretreated with 2% CHX in accordance with the *in vitro* specimen preparation protocol. Following adhesive application, the cavities were restored with a hybrid resin composite (Filtek Z250) as with the *in vitro* specimens, and finishing and polishing procedures were performed after 24 hours. Half of the patients' teeth were extracted after 24 hours for evaluation of immediate bond strength, while the remaining teeth were periodically monitored and extracted six months later. Extracted teeth were stored in distilled water at room temperature until bond strength evaluation.

Microtensile Bond Test

Restored teeth were longitudinally sectioned across the bonded interface using a water-cooled diamond saw in a precision cutting machine (Micracut 201,

Table 2. Microtensile Bond Strength Values (in MPa) and SD (±) of Tested Adhesive Systems (n=5)

Adhesive Systems	Time	Chlorhexidine	*In Vitro* Mean ± SD	*In Vivo* Mean ± SD
Adper Single Bond 2 (SB)	Immediate	CHX (−)	36.13 ± 2.46	28.45 ± 2.25
		CHX (+)	31.36 ± 1.29	24.37 ± 1.32
	Aged	CHX (−)	17.60 ± 1.71	16.33 ± 0.67
		CHX (+)	23.91 ± 1.23	19.87 ± 0.66
Clearfil SE (SE)	Immediate	CHX (−)	35.38 ± 2.26	27.13 ± 2.04
		CHX (+)	32.94 ± 1.72	24.14 ± 1.07
	Aged	CHX (−)	17.97 ± 1.31	16.20 ± 0.52
		CHX (+)	23.68 ± 1.67	20.11 ± 1.36
Clearfil S^3 (S^3)	Immediate	CHX (−)	30.23 ± 1.20	24.95 ± 1.01
		CHX (+)	27.5 ± 1.54	22.98 ± 0.82
	Aged	CHX (−)	14.36 ± 1.16	12.81 ± 1.00
		CHX (+)	19.83 ± 0.78	16.08 ± 0.68
Adper Prompt-L-Pop (PLP)	Immediate	CHX (−)	27.11 ± 0.96	24.18 ± 0.22
		CHX (+)	23.69 ± 1.12	20.40 ± 0.71
	Aged	CHX (−)	12.96 ± 0.49	10.84 ± 1.16
		CHX (+)	17.20 ± 0.65	14.02 ± 0.56

MetLab Inc, Niagara Falls, NY, USA) to obtain bonded sticks with a cross-sectional area of approximately 1 mm^2. The exact dimension of each beam was measured using a digital caliper. Slabs sectioned from the center of each composite restoration were selected. Three beams were selected from each tooth, resulting in 15 beams for each subgroup. Each beam was attached to the test apparatus with a cyanoacrylate adhesive and stressed to failure under tension using a universal testing machine (Micro Tensile Tester, Bisco, Schaumburg, IL, USA) with a 50 kgf load cell and a crosshead speed of 1 mm/min.

The fractured specimens were examined using a stereomicroscope (SZ 61, Olympus, Tokyo, Japan) at 25× magnification. The failure modes were classified and recorded as cohesive (failure within the composite resin or within dentin), adhesive (failure across the bonding interface), or mixed failure.

Scanning Electron Microscopic Evaluation

Two specimens were randomly selected from each group to evaluate the effect of CHX application on resin-dentin interface. As a preparation for scanning electron microscopic evaluation, specimens were first etched with 37% phosphoric acid for 15 seconds followed by 30 seconds rinsing with water. The specimens were fixed in 10% neutral buffered formalin for 24 hours and rinsed in running water for 15 minutes. Then, the specimens were dehydrated in ascending grades of ethanol (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes, and 100% for 60 minutes) and thereafter, were immersed in hexamethyldisilazane for 10 minutes. Finally, the specimens were placed on a filter paper inside a covered glass vial, and air-dried at room temperature for 12 hours. The specimens were sputter-coated with gold and examined through scanning electron microscopy (SEM).

Statistical Analysis

Statistical analysis of the data was made using four-way analysis of variance (ANOVA). Bonferroni test was utilized for pair-wise comparisons. Results of failure modes were subjected to nonparametric analysis using Pearson chi-square test. All results were analyzed with SPSS 18.0 for Windows (SPSS Inc, Chicago, IL, USA), with the level of significance set at $p<0.05$.

RESULTS

The μTBS values of test groups are presented in Table 2 as means and standard deviation. All tested variables (type of adhesive, CHX pretreatment, aging, and *in vivo*/*in vitro* testing), significantly influenced the dentin bond strength ($p<0.05$, four-way ANOVA).

For all tested adhesives, the immediate bond strength values were significantly higher than "aged" ones ($p=0.00$). While CHX pretreated groups showed significantly lower immediate bond strength values ($p<0.05$), they yielded higher bond strength values in aged samples ($p<0.05$) (Table 3). There were statistically significant differences between *in vitro* and *in vivo* immediate dentin bond strength

Table 3. *Results for the Comparison of Means (MPa) of the CHX (+) vs CHX (–) Within the Levels of the Test Conditions, Adhesive Systems, and Time (α=0.05).*

Test Condition	Adhesive Systems	Time	*p*-Value
In vitro	SB	Immediate	0.000
		Aged	0.000
	SE	Immediate	0.003
		Aged	0.000
	S^3	Immediate	0.001
		Aged	0.000
	PLP	Immediate	0.000
		Aged	0.000
In vivo	SB	Immediate	0.000
		Aged	0.000
	SE	Immediate	0.000
		Aged	0.000
	S^3	Immediate	0.003
		Aged	0.000
	PLP	Immediate	0.000
		Aged	0.000

Table 4. Results For The Comparison of Means (MPa) of The *In Vitro* vs *In Vivo* Test Conditions Within The Levels of The Adhesive Systems, Time, And CHX Application

Adhesive Systems	Time	CHX	*p*-Value
SB	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.122*
		(+)	0.000
SE	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.078*
		(+)	0.000
S^3	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.116*
		(+)	0.000
PLP	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.059*
		(+)	0.000

* *No statistically significant difference,* $p>0.05$.

values for all tested adhesives regardless of CHX pretreatment ($p<0.05$). While there were significant differences between *in vitro* and *in vivo* aged CHX treated samples ($p<0.05$), no difference was observed in non-CHX treated samples ($p>0.05$) (Table 4). The fracture mode was predominantly adhesive and mixed, and was similar among the experimental groups ($p>0.05$).

In Vitro Specimens

In the absence of CHX pretreatment, immediate and aged dentin bond strength values were similar for SB and SE, and for S^3 and PLP ($p>0.05$), with the immediate and aged dentin bond strength of SB and SE being significantly higher than S^3 and PLP ($p<0.05$). CHX pretreatment caused significant differences in immediate dentin bond strength in all groups except between SB and SE ($p=0.336$). Significant differences were also observed in "aged" CHX-pretreated groups, except between SB and SE, and S^3 and PLP ($p>0.05$) (Table 5).

In Vivo Specimens

In experimental groups that did not receive prior CHX treatment, results were similar as with their *in vitro* counterparts, with the exception of insignificance between SE and S^3. For CHX-pretreated samples, immediate bond strength values were similar among groups with the exception of PLP, yielding lower dentin bond strength compared with other groups ($p<0.05$). In aged CHX-pretreated groups, results were similar as with their *in vitro* counterparts (Table 5).

Figures 1 through 4 demonstrate representative scanning electron micrographs of the resin-infiltrated zone. Regardless of the type of adhesive, testing condition (ie, *in vivo*/*in vitro*), or CHX pretreatment, all specimens showed a well-defined, continuous hybrid layer that slightly varied in thickness along the resin-dentin interface.

DISCUSSION

While many studies have investigated the effects of CHX pretreatment on dentin bond strength, controversy still exists regarding whether CHX decreases immediate bond strength.[12,16,19,21-24] In the present study, CHX treatment caused lower immediate dentin bond strength values in all test groups. In line with the present results, Campos and others[22] observed that 2% CHX caused a decrease in immediate dentin bond strength values of self-etch adhesives, Clearfil SE Bond, and Clearfil S^3 Bond. In the same study, however, CHX application had no influence on the immediate bond strength values of the tested etch-and-rinse adhesives. Other studies utilizing different self-etch or etch-and rinse adhesives have reported that CHX has no effect on immediate bond strength values.[12,19,23-26] In anoth-

Table 5: *Results for the Comparison of Means (MPa) of the Adhesive Systems Within the Levels of the Test Conditions, CHX Application, and Time*

Adhesive Groups	*In Vitro p*-Value				*In Vivo p*-Value			
	CHX(−) Immediate	CHX(+) Immediate	CHX(−) Aged	CHX(+) Aged	CHX(−) Immediate	CHX(+) Immediate	CHX(−) Aged	CHX(+) Aged
SB vs SE	1.000*	0.336*	1.000*	1.000*	0.657*	1.000*	1.000*	1.000*
SB vs S^3	0.000	0.000	0.001	0.000	0.002	0.557*	0.000	0.000
SB vs PLP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SE vs S^3	0.000	0.000	0.000	0.000	0.259*	0.955*	0.000	0.000
SE vs PLP	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000
S^3 vs PLP	0.070*	0.000	0.217*	0.227*	0.735*	0.016	0.107*	0.077*

** No statistically significant difference, p>0.05.*

er study, CHX was found to preserve the bonding durability of an etch-and-rinse adhesive (Single Bond) but was unable to maintain a stable bond of a one-step self-etch adhesive system (GBond).[27] Because CHX generally inhibits MMPs without impairing dentin bond strength,[28] the benefits of CHX pretreatment might be expected over the course of time. Manfro and others[21] demonstrated the effectiveness of CHX in preventing the degradation of the adhesive interface in primary dentin. In their study, they found no significant reduction in dentin bond strength in aged samples, while in the absence of CHX treatment, significant reductions were observed. The present results corroborate with those of previous studies reporting that CHX application has no adverse effect on aged dentin bond strength values. Further, the present study demonstrates that CHX treatment leads to higher aged dentin bond strength compared with non-CHX-treated groups. Therefore, the first null hypothesis should be rejected. It has been proposed that the use of 2% chlorhexidine before application of adhesive resins reduces the deterioration of hybrid layers following exposure to water.[29] In the present study, the higher bond strength values obtained in aged CHX-treated samples might be related with the inhibition effect of CHX on MMPs that had been activated by the acidity of the tested adhesives. As known, mild acids have a potential to activate MMPs, and particularly, pH values ranging from 2.3 to 5 are effective in activating gelatinases.[30] The self-etch adhesive used in this study has a pH value of 2.4 and is, thus, capable of enhancing dentin proteolytic activity without denaturation of the enzymes. The observation of increased aged bond strength after CHX pretreatment has been demonstrated previously, utilizing an etch-and-rinse and a self-etch adhesive.[31]

For the experimental groups that did not receive CHX-pretreatment, our results confirm previous studies that reported reductions in the dentin bond strength of different self-etch and etch-and-rinse adhesives after aging.[32-37] For CHX-pretreated groups, however, establishing correlations between the present results and those of previous reports might be more complicated. It has been shown that CHX has no significant effect on bond strength stability, and that there was a decrease in bond strength over a six-month period of water storage.[37] Further, in an 18-month clinical study comparing the performance of an etch-and-rinse and self-etch adhesives in noncarious cervical lesions with and without CHX pretreatment,[38] the authors concluded that CHX did not influence the performance of the tested adhesives. Undoubtedly, clinical retention is

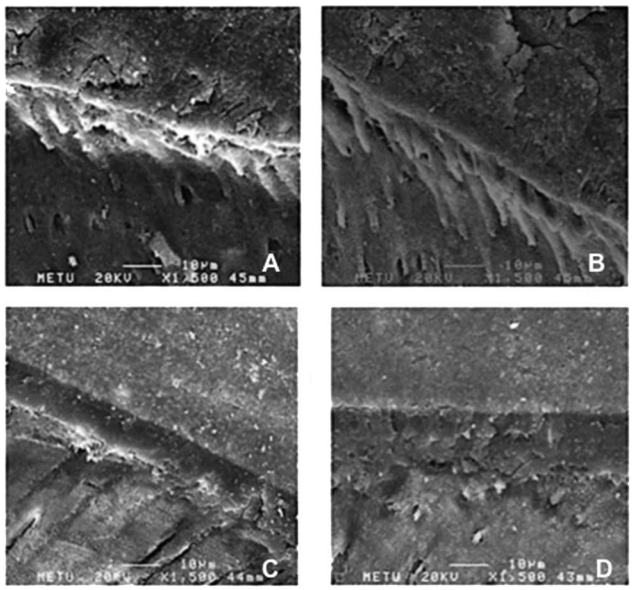

Figure 1. *SEM images of composite-dentin interface with Adper Single Bond 2. (A): Immediate and CHX (+). (B): Immediate and CHX (–). (C): Aged and CHX (+). (D): Aged and CHX (–).*

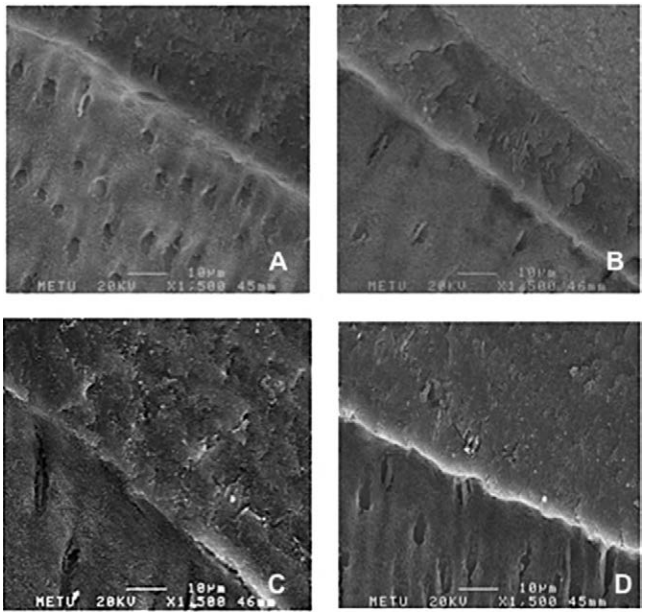

Figure 2. *SEM images of composite-dentin interface with Clearfil SE Bond. (A): Immediate and CHX (+). (B): Immediate and CHX (–). (C): Aged and CHX (+). (D): Aged and CHX (–).*

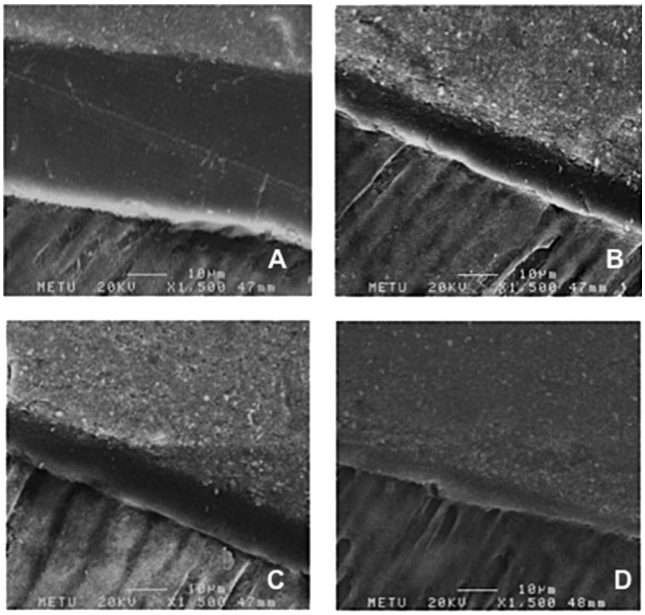

Figure 3. *SEM images of composite-dentin interface with Clearfil S^3. (A): Immediate and CHX (+). (B): Immediate and CHX (–). (C): Aged and CHX (+). (D) Aged and CHX (–).*

affected by many other factors, but those results should be considered when drawing conclusions.

Although clinical trials remain the ultimate instrument, preclinical screenings of materials are still important. However, it is still not fully understood whether there is a relationship between laboratory data and clinical outcomes and also whether the clinical performance is predictable in the lab. To date, only a few studies have compared the behavior of dental biomaterials under both clinical and laboratory conditions.[11,37,39-41] The present results indicated differences in the bond strength values of CHX-pretreated groups, with higher bond strength values obtained in *in vitro* test results. This can be expected , particularly due to the insufficient simulation of oral conditions with thermocycling. Thermocycling is a common procedure to predict how a material would perform over time clinically. Although it is one of the most popular artificial aging methods, there is no evidence to indicate that thermocycling would create specimens equivalent to those fabricated *in vivo*. Patient-related factors constitute the difference between the laboratory and the clinical study. Comparing the bonding effectiveness of the etch-and-rinse adhesive to the two-step self-etch adhesive, it is evident that both adhesives bonded similarly under *in vivo* and *in vitro* conditions in non-CHX treated samples. Immediate bond strength values of one-step and all-in-one self-etch adhesives, Clearfil S^3 Bond and Adper Prompt-L-Pop, were also similar. Therefore, the second null hypothesis should be partly accepted.

In the present study, the bond strength values of Adper Single Bond and Clearfil SE Bond were significantly higher than the tested one-step self-etch adhesives. Several studies have shown that etch-and-rinse adhesives yield bonding values supe-

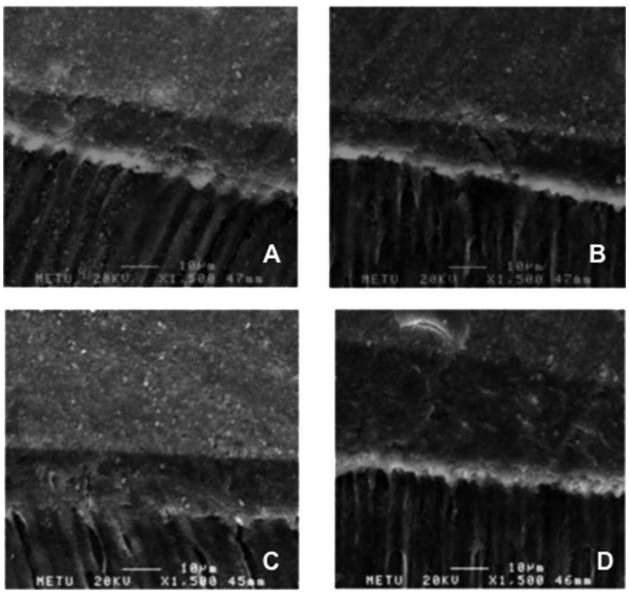

Figure 4. *SEM images of composite-dentin interface with Prompt-L-Pop. (A): Immediate and CHX (+). (B): Immediate and CHX (–). (C): Aged and CHX (+). (D): Aged and CHX (–).*

rior to those of one-step self-etch adhesives. Perdigao and others[42] demonstrated that Adper Single Bond and Clearfil SE Bond showed similar bonding values that were also higher than one-step self-etch adhesives. Indeed, in several studies, Adper Single Bond and Clearfil SE Bond have shown significantly higher bond strength values compared with other different adhesives.[34,43] The ethanol and HEMA content of Single Bond enables high wettability, whereas 10-MDP in Clearfil SE Bond binds to calcium salts that maintain a stable bonding interface.[34] Further, the relatively higher concentration of camphorquinone in Clearfil SE Bond improves its polymerization rate.[44]

In the present study, the lowest bond strength values were obtained in aged one-step self-etch adhesives. Presumably, such levels of bond strength may result from the high acidity of these adhesives that degrades the collagen matrix stabilization.[45] It may be possible to speculate that additional factors, not possible to show with the present methodology, such as resin monomers with low conversion rate[44] and occurrence of water trees,[34,45] may have contributed to these results. As bond strength values differ according to the tested adhesive systems, the third null hypothesis should also be rejected.

Adhesive and mixed failure types have been observed in a majority of microtensile bond strength studies, irrespective of CHX pretreatment.[20-22,31,34] Osorio and others[34] reported that while mixed failure types were observed after immediate bond strength testing, adhesive failures were prominent in aged groups subjected to debonding forces. In the present study, predominance of adhesive/mixed failure was observed in all groups with a lack of statistical significance. In resin-dentin interface evaluation by SEM, the hybrid layer was found to be intact and mostly continuous in all tested samples.

Additional long-term studies might be necessary to determine the long-term effects of CHX on the bond strengths of different adhesive systems.

CONCLUSIONS

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

1) The immediate bond strength values of all tested adhesives were significantly higher than "aged" ones.
2) The application of CHX caused lower immediate and higher aged bond strength values.

Regulatory Statement

This study was conducted in accordance with all of the provisions of the local human subjects oversight committee guidelines and policies. The approval code for this study is LUT 09/72-21. This study was conducted at Hacettepe University.

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Conflict of Interest

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

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Clinical Performance of Composite Restorations with Resin-modified Glass Ionomer Lining in Root Surface Carious Lesions

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Clinical Relevance

The application of cavity lining material did not affect clinical performance, particularly marginal adaptation rate, over an 18-month period.

SUMMARY

Objective: The purpose of this study was to evaluate the clinical performance of composite restorations in root surface carious lesions with or without resin-modified glass ionomer lining.

Methods and Materials: The sample consisted of 25 female and 14 male patients. A maximum of four lesions were included for each patient. After caries removal, the depth, length, and width of the cavity were measured. Lesions in the same patient were randomly divided into two groups, and the dentin surfaces were either lined with resin-modified glass ionomer liner (Glass Liner II) or left as they were. Self-etch adhesive (All Bond SE) was applied and cured for 20 seconds. All cavities were restored with nanohybrid anterior composite resin (Clearfil Majesty Esthetic). Two experienced clinicians evaluated the marginal adaptation (retention) rate, anatomic form, secondary caries, sensitivity, and marginal staining of restorations at the end of the first week and at six, 12, and 18 months posttreatment. The data were statistically analyzed using the Chi-square and two-way repeated measures tests.

Results: At the end of 18 months, a total of five lined and three unlined restorations were lost. There was no significant relationship between marginal adaptation and cavity lining at six, 12, and 18 months ($p>0.05$). Although marginal stainings of restorations were mostly localized, the total number of localized or generalized discolored restorations increased with time ($p<0.001$). There was a statistically significant relationship between marginal staining and smoking ($p>0.05$). There was no significant relationship between marginal staining and frequency of toothbrushing at six, 12, and 18

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months (p=0.286, p=0.098, and p=0.408, respectively).

Conclusion: Within the limitations of this study, both restorative applications were accepted as clinically appropriate.

INTRODUCTION

The etiology of root surface caries is multifactorial, with microbiological factors playing a critical role. The cervical margins of root surface carious lesions are usually located in the cementum or dentin, making it more susceptible to bacterial invasion (microleakage). Microleakage can lead to problems such as marginal staining, secondary caries, and pulpal inflammation.[1] The pulp requires protection from further bacterial invasion of the carious process and chemical protection from the overlying restorative materials.[2] This may be achieved by placement of a biocompatible liner on the cavity floor.[3] Lining with resin-modified glass ionomers (RMGICs) has been shown to lead to clinical success and also to promote a dramatic reduction in the number of cariogenic bacteria.[4] Resilient liners, such as RMGICs, are capable of absorbing the polymerization contraction stress of the overlying resin composites.[5,6] Contraction stress is one of the main causes of microleakage and often leads to postoperative sensitivity, marginal staining, and secondary caries.[5,6] An *in vitro* study[7] reported the lowest percentage of marginal gap with RMGIC liners.

Materials such as resin composites are widely used for the restoration of root surface caries as they do not require excessive removal of sound tooth structure and have a low modulus of elasticity. Self-etching adhesives are often used for bonding of composite restorations of root surface caries as they are easy to manipulate. On the other hand, some authors[8,9] reported that application of adhesives in deep cavities resulted in acceptable levels of biocompatibility. However, the intensity of the pulpal response depended upon the thickness of the remaining dentin. These views motivated clinicians to reevaluate the use of liners and adhesives.

The aim of this randomized, controlled clinical trial was to evaluate the clinical performance of a nanohybrid composite resin restoration in carious root surface lesions lined with and without a RMGIC liner. The cavity size, toothbrushing, and smoking habits of patients were also included as additional observations.The null hypothesis tested was that there is no difference between the two restorative systems with respect to marginal adaptation (retention), marginal staining, secondary caries, and postoperative sensitivity.

METHODS AND MATERIALS

Thirty-nine nonhospitalized volunteer patients who presented with root surface carious lesions at the Restorative Dental Clinic were randomly selected for this study. Their ages ranged between 18 and 67 years, with the mean age being 39.62 ± 13.85 years.

The local institutional review board approved this clinical trial prior to commencement. The volunteers were informed about the conditions and objectives of the study and were asked to provide informed consent.

A paired-tooth design was used for this study. Patients who were under the age of 18 years or who had complex medical histories, severe or chronic periodontitis, extreme carious activity, heavy bruxism, very deep or superficial carious lesions, or previously restored and abutment teeth were excluded from the study. Lesions were classified as inactive if their surface was leathery, shiny, or not covered with plaque.

A single operator carried out all restorative procedures, and the lesion surface was pumiced before the intervention. Moisture control was carried out with the help of cotton rolls and a saliva ejector. Cavity preparation was completed with a diamond bur, and the carious lesion was removed with the help of a steel bur. The enamel margins were beveled but no mechanical retention was performed. The depth, width, and length of the cavity were measured and recorded by two experienced and calibrated clinicians using a periodontal (WHO 973/80-Martin, Solingen, Germany) explorer. In each patient, two or four root surface carious lesions were randomly restored according to two experimental protocols. A coin was tossed and depending on the outcome cavities were either lined or left as they were. The liner was placed and light-cured for 20 seconds and the enamel walls were not sealed with the lining material. Thereafter, two separate coats of All Bond SE (one-step self-etch adhesive) were applied using a microbrush for 15 seconds per coat (no light-cure between coats) for all cavities, air-dried for at least 10 seconds, and then LED light-cured for 10 seconds (Led Max 5; Hilux, Benlioglu Dental, Ankara, Turkey) set at 500-700 mW/cm^2 intensity. The materials used in this study are presented in Table 1.

Both groups were restored with the nanohybrid composite (Clearfil Majesty Esthetic) using the

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

Materials	Composition	Manufacturer	Batch No.
All bond SE	Part I—ethanol, sodium benzene sulfinate dehydrate Part II—Bis (glyceryl 1,3 dimethacrylate)–phosphate; hydroxyethyl methacrylate biphenyl dimethacrylate	Bisco Inc, Schaumburg IL, USA	0900004181 0900004182
Glass Liner II	Liquid: polycarbonic acid-water Powder: calcium-aluminum-fluoro-silicate, barium glass	WP Dental, Barmstedt, Germany	120298
Clearfil Majesty Esthetic	Matrix: Bis-GMA, hydrophobicaromatic dimethacrylates, and hydrophobicaliphatic dimethacrylates, DL-camphorquinone	Kuraray, Tokyo, Japan	00014C

Abbreviation: Bis-GMA, bisphenol A diglycidyl ether dimethacryalate.

incremental technique with a flat-faced condenser. Each resin composite layer was light-cured for 20 seconds with the same LED light-curing unit. All restorations were finished with extra-/ultrafine composite finishing burs (Diatech Dental AC, Heerbrugg, Switzerland) and polished with discs (SwissFlex; Diatech Dental AC) and Enhance PoGo Complete Kit (Dentsply, Addlestone, York, PA, USA).

A double-blind study design was used, and two calibrated clinicians (other than the operator) evaluated all restorations. Direct intraoral clinical examination was carried out by two calibrated examiners. The quality of the restorations was evaluated according to marginal adaptation (retention), anatomical form, caries in adjacent tooth structure, and caries at the cavosurface margin (Table 2). The criteria developed by Haveman and others[10] were modified by adding sensitivity and marginal staining scores.

The sensitivity score was based on the subjective symptoms of the patient to cold air (yes/no), while staining of restorations was determined visually as being localized/generalized. Marginal staining was defined as localized if there was discoloration on less than half of the circumferential margin or defined as generalized if there was discoloration on more than half of the circumferential margin.[11]

The width, length, and depth (in millimeters) of the cavities were grouped before statistical analysis. Toothbrushing frequencies were identified as once or twice a day. Smokers were also divided into the following three groups: nonsmoker, 1-10 cigarettes per day, and more than 10 cigarettes per day.

Restorations were evaluated at baseline and again at six, 12, and 18 months posttreatment. The baseline rating was carried out one week posttreatment. Chlorhexidine (Kloroben, Drogsan, Turkey) mouth-rinse was prescribed for five days, and standard toothpaste (Colgate Total; 1450 ppm sodium fluoride [NaF] 0.3% triclosan/copolymer/ 0.22% NaF) was advised for daily use.

Table 2: *Evaluation Criteria (1)*

Score	Marginal Adaptation (Retention)
0	The restoration appears to adapt closely to the surface of the tooth with no crevice formation. An explorer either did not catch when drawn along the margin or only did so when passed in one direction.
1	An explorer caught lightly when run in both directions, and there was visible evidence of early crevice formation. Dentin was not visible.
2	An explorer got caught in both directions and penetrated a marginal crevice. There was visible evidence of crevice formation. However, the dentin was not visible.
3	The crevice had sufficient depth to expose the dentin. The restoration required replacement.
4	The restoration was fractured or lost.
	Anatomical Form
0	The restoration was continuous with the existing tooth anatomy.
1	The restoration was not continuous with the existing tooth anatomy, but no dentin was exposed. The restoration was clinically acceptable.
2	The restoration was not continuous with the existing tooth anatomy and required replacement.
	Caries in Adjacent Tooth Structure
0	Caries was not present within 3 mm of the border of the restoration.
1	Caries was present within 3 mm of the border of the restoration.
	Caries at the Cavosurface Margin
0	No caries was present on the cavosurface margin.
1	Caries was present on the cavosurface margin.
	Sensitivity
Yes	Sensitive to cold air
No	Not sensitive to cold air
	Marginal Staining
0	No staining
1	Localized staining
2	Generalized staining

Table 3: *The Distribution of Teeth According to the Location*

Cavity Lining	Dental Arch	Tooth Distribution	n	%
Lined restorations (n=50)	Upper (n=26)	Anterior	20	20.0
		Premolar	5	5.0
		Molar	1	1.0
	Lower (n=24)	Anterior	10	10.0
		Premolar	11	11.0
		Molar	3	3.0
Unlined restorations (n=50)	Upper (n=22)	Anterior	17	17.0
		Premolar	5	5.0
		Molar	—	—
	Lower (n=28)	Anterior	13	13.0
		Premolar	10	10.0
		Molar	5	5.0
Total			100	100

Photographs were taken at every step of restoration and at the recalls.

Statistical Analysis

All statistical analyses were performed using the SPSS 21.0 package. Pearson Chi-square tests were used to analyze differences between the two restorative procedures at the 5% significance level. Intra-restorative procedure comparisons of baseline and at six and 12 months posttreatment were also performed. Differences in marginal adaptation and marginal staining of restorations over time were analyzed using the two-way repeated measures test.

RESULTS

In this study there were 100 restorations in 39 patients. Twenty-eight patients received two restorations each, and 11 patients received four restorations each. Most of the carious lesions were active. The distribution of the teeth based on their location in the dental arch is shown in Table 3.

All lesions were deeper than 1 mm. The mean cavity depth was 2.18 ± 0.72 mm, mean occluso-gingival width was 3.53 ± 1.03 mm, and mean length was 4.75 ± 1.68 mm.

There were no statistically significant differences in cavity depth, length, and width and marginal adaptation as well as marginal staining rates at six, 12, and 18 months ($p>0.05$).

At baseline all restorations were adapted closely to the tooth surface, continuous with the existing tooth anatomy, and sensitivity, staining, and caries were not present. At the end of six months two lined restorations were lost. At the end of 12 months, three patients lost one of the restorations and one patient lost both restorations. One patient's tooth was extracted because of acute periodontal disease, and two patients chose crown restorations. At the end of 18 months, one patient had lost one of the restorations and one patient did not respond for recall (Table 4).

According to the results of the two-way repeated measures test, cavity lining had no effect on marginal adaptation ($p=0.566$), but changes in marginal adaptation, which was decreased over time, were significant ($p=0.0001$). According to the results of the Chi-square test, there was no significant relationship between cavity lining and marginal adaptation at six, 12, and 18 months ($p=0.187$, $p=0.557$, and $p=0.675$, respectively).

In terms of anatomic forms, all remaining restorations were continuous with the existing tooth anatomy.

No caries in the adjacent tooth structure or at the cavosurface margin were observed in either restoration group at six, 12, and 18 months, and sensitivities disappeared after the restorative rehabilitation.

At the end of six months, four teeth showed marginal staining; at the end of 12 months, 12 teeth showed staining; and at the end of 18 months 28 teeth showed marginal staining (Table 5). Two-way repeated measures test showed that there was no significant relationship between cavity lining and marginal staining ($p=0.301$). Marginal staining of the restorations was mostly localized in nature, and according to the results of the two-way repeated measures test the overall number of discolored restorations (localized and generalized) increased with time ($p=0.0001$).

Approximately 43.6% of the sample were smokers, and according to the two-way repeated measures test there was a statistically significant association between marginal staining and smoking ($p=0.038$).

Frequency of toothbrushing increased after the restorative treatment but reversed within 12 months, a finding that was insignificant according to the two-way repeated measures test ($p=0.068$). According to the Chi-square test, there was no significant relationship between marginal staining and frequency of toothbrushing at six, 12, and 18 months ($p=0.286$, $p=0.098$, and $p=0.408$, respectively).

DISCUSSION

Most root surface carious lesions do not require restorative rehabilitation. Accessible superficial le-

Table 4: *Marginal Adaptation Scores at Baseline and at Six, 12, and 18 Months*

Recall	Marginal Adaptation Scores	Cavity Lining				Total
		Unlined		Lined		
		n	%	n	%	n
Baseline	0	50	100	50	100	100
	1	—	—	—	—	
	2	—	—	—	—	
	4	—	—	—	—	
	Not applicable[a]	—	—	—	—	—
6 mo	0	46	92	42	84	88
	1	4	8	6	12	10
	2	—	—	—	—	—
	4	—	—	2	4	2
	Not applicable	—	—	—	—	—
12 mo[b]	0	34	68	30	60	64
	1	9	18	13	26	22
	2	—	—	—	—	
	4	2	4	3	6	5
	Not applicable	5	10	4	8	9
18 mo[c]	0	23	46	22	44	45
	1	15	30	17	34	32
	2	1	2	1	2	2
	4	1	2	—	—	1
	Not applicable	10	20	10	20	20
Total		50	100	50	100	100

[a] *Lost restorations, extracted teeth, or no attendance were not evaluated.*
[b] *At the end of one year, one patient did not return for recall. Three patients lost one of the restorations; another patient lost both of the restorations, whereas another patient's tooth has been extracted and two patients chose crown restoration.*
[c] *At the end of 18 months, one patient had lost one of the restorations and one patient did not return for recall.*

Table 5: *Marginal Staining at Baseline and at Six, 12, and 18 Months*

Recall	Staining	Cavity Lining			
		Unlined		Lined	
		n	%	n	%
Baseline	No staining	50	100	50	100
	Localized staining	—	—	—	—
	Generalized staining	—	—	—	—
	Not applicable	—	—	—	—
6 mo	No staining	49	98	45	90
	Localized staining	1	2	3	6
	Generalized staining	—	—	—	—
	Not applicable	—	—	—	—
12 mo	No staining	40	80	33	66
	Localized staining	3	6	9	18
	Generaliszd staining	—	—	1	2
	Not applicable	7	14	7	14
18 mo	No staining	27	54	24	48
	Localized staining	12	24	14	28
	Generalized staining	—	—	2	4
	Not applicable	11	22	10	20

sions can be made caries-free easily using hand instruments and finishing and polishing burs. Hard and leathery areas can be treated with chlorhexidine, topical fluoride application, fluoride dentifrices, and saliva.[12] Turssi and others[13] showed that saliva substitutes may induce partial remineralization in preformed caries-like lesions in the root dentin. If root surface caries are deeper than 1 mm, restorative treatment with different materials, such as amalgam, composite resin, or glass ionomer cement, is required.[14,15]

Glass ionomers (GICs) are used as a liner/base and restorative material because of their chemical bonding to the tooth substrate, long-term fluoride ion release, low coefficient of thermal expansion, acceptable esthetic quality, and biocompatibility.[16] GIC adheres strongly to dentin as the polyacrylate ions attach irreversibly to the surface of hydroxyapatite by displacing existing phosphate ions. GIC also provides micromechanical attachment to the composite.[17-19]

Banomyong and others[20] used total or self-etching adhesives and reported that the qualities of the posterior resin restorations were not significantly affected by the placement of GIC lining, regardless of the adhesive used, after one year. This corresponds with the results of the present study, in which no statistically significant differences in marginal adaptation rates were found between the lined and unlined restorations.

However, to the best of our knowledge, there are no recent clinical studies that involved restoration of root surface caries with composite resin. In 1990, Levy and Jensen[21] compared restoration of root surface caries using GIC or microfilled composite resin and reported a 73% success rate at the end of 24 months with the latter. In the present study, a success rate of approximately 90% was achieved. This higher success rate could be explained by the use of adhesives, as the previous study relied only on mechanical retention and a six months' shorter time period than was used in the present study.

As there is a lack of studies investigating restoration of root surface caries, we compared our results with the marginal adaptation scores of restorations performed on noncarious cervical lesions (NCCLs). Several studies investigated the treatment of NCCLs with bevel using different types of self-etch adhe-

sives and reported a greater than 80% success rate for marginal adaptation of restorations at the end of 12 to 24 months.[22,23] Loguercio and others[24] used one-step and two-step All Bond SE self-etch adhesives and reported an 84.8% success rate with the former at the end of 24 months. The findings of the latter study were in accordance with those of the present study, which used the same adhesive system.

It has been suggested[25] that the retention of an adhesive restoration depends on the marginal adaptation capacity as well as the viscoelastic properties of the restorative system. Several factors can bring about dimensional changes to these restorative materials, including thermal changes and water absorption.[26] In addition to using adhesive materials for marginal adaptation, retention can be better achieved by preparing the cavity such that the adhesion surface increases.[27] However, the results of the present study showed no statistically significant differences in cavity depth, length, and width and marginal adaptation rates at six, 12, and 18 months. These results do not support that increasing the size of the preparation is beneficial.

Despite the initial color match being favorable, the restoration margins may show staining over time. Kubo and others[26] compared two self-etch adhesive systems on NCCLs. They reported that at the end of two years, 20% of the restorations had slight marginal staining. Loguercio and others[24] reported that marginal staining was observed in a few restorations at 24 months. Perdigão and others[28] indicated that the number of satisfactory color ratings significantly decreased after one year with different one-step self-etch adhesive systems. In agreement with these studies, our results showed that marginal staining increased over time (5.5% at six months, 24.4% at 12 months, and 28% at 18 months).

Peumans and others[22] conducted a study on the treatment of NCCLs using composite resin and reported superficial localized margin discoloration, which was observed significantly more often in smokers, in correlation with the results of this study. In addition, regarding the effect of clinical covariables (size of lesion and toothbrushing), no correlation was found, as was the case in this study.

The frequency of root caries among young age groups was explored in this study and may partially reflect the poor oral health of Turkish people, as previously reported by Gökalp and Dogan[29] in the 2004 National Oral Health Survey. The prevalence rate of root caries in the 35-44-year-old age group was 20.1%. Most of the patients claimed that they brushed their teeth twice a day, which is sufficient for daily oral hygiene. However, these patients still presented with root surface caries. It has been suggested that though the frequency of brushing may have been enough, its duration was short, and the patient's technical knowledge was inadequate or wrong. This may be responsible for the higher incidence of root caries in younger individuals. At the beginning of this study, patients were informed about toothbrushing techniques and their importance. They were asked to brush their teeth at least twice per day. Frequency of toothbrushing was seen to increase after restorative procedures but declined after 12 months.

De Moor and others[30] reported a significantly higher incidence of recurrent caries in glass ionomer and composite resin restorations of root caries in xerostomic irradiated head and neck cancer patients after 24 months. In contrast, Hu and others[31] reported no active recurrent caries with glass ionomer restorations in their radiation-induced root surface caries study. Differences may originate from general systemic disorders of the patients. In this study, no caries were seen within the borders of the restoration. The dentifrice recommended in this study may have increased fluoride levels and thereby prevented secondary caries.[32,33]

Tooth sensitivity before restorative rehabilitation may be reduced with the help of self-etch adhesive systems. Perdigão and others[28] reported that postoperative sensitivity to air improved significantly with different self-etch adhesive systems. In the present study we found a reduction in tooth sensitivity.

The advantages of this clinical study include a paired-tooth study design, randomization, relatively high restoration number, adequate follow-up (will be continued over the duration of four successive years), and appropriate statistical analysis. Moreover, a complete description of the study methodology and results has been provided to allow comparison with other root surface caries clinical trials with similar study designs. The null hypothesis was accepted, as the lining of the restoration had no effect on the marginal adaptation, marginal staining, secondary caries, and postoperative sensitivity.

CONCLUSIONS

Within the limitations of this study, the application of cavity lining material did not affect clinical

performance, particularly marginal adaptation rate. All Bond SE exhibited good performance; both restorative techniques were considered clinically appropriate at 18 months.

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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 Hacettepe University. The approval code for this study is 11/54-8.

Conflict of Interest

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

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Laboratory Research

Effect of Adhesive Cementation Strategies on the Bonding of Y-TZP to Human Dentin

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Clinical Relevance

A 10-methacryloxydecyl dihydrogen phosphate monomer–based universal adhesive primer is a viable alternative to air-abrasion surface conditioning when bonding zirconia to dentin.

SUMMARY

This study evaluated the effects of different adhesive strategies on the adhesion of zirconia to dentin using conventional and self-adhesive cements and their corresponding adhesive resins. The occlusal parts of human molars (N=80) were sectioned, exposing the dentin. The teeth and zirconia cylinders (N=80) (diameter=3.4 mm; height=4 mm) were randomly divided into eight groups according to the factors "surface conditioning" and "cement type" (n=10 per group). One conventional cement (CC: RelyX ARC, 3M ESPE) and one self-adhesive cement (SA: RelyX U200, 3M ESPE) and their corresponding adhesive resin (for CC, Adper Single Bond Plus; for SA, Scotchbond Universal Adhesive-SU) were applied on dentin. Zirconia specimens were conditioned either using chairside (CJ: CoJet, 30 μm, 2.5 bar, four seconds), laboratory silica coating (RC: Rocatec, 110 μm, 2.5 bar, four seconds), or universal primer (Single Bond Universal-UP). Nonconditioned groups for both cements acted as the control (C). Specimens were stored in water (37°C, 30 days) and subjected to shear bond strength (SBS) testing (1 mm/min). Data (MPa) were analyzed using two-way analysis of variance and a Tukey test (α=0.05). While surface conditioning significantly affected the SBS values (*p*=0.0001)

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(C<RC=CJ=UP), cement type did not (p=0.148) (CC=SA). The interaction terms were significant (p=0.014). Failure types were predominantly adhesive. Air-abrasion and the use of the universal primer improved the bond strength of zirconia to dentin compared to the control group, regardless of the type of resin cement used.

INTRODUCTION

Zirconia-based ceramics have superior mechanical properties compared to the other ceramic materials, such as glass-based ceramics.[1,2] This material has been used as the framework for crowns and fixed dental prostheses (FDPs) during the last two decades and, more recently, has been indicated for monolithic crowns or FPDs.[3] Since zirconia is totally crystalline and is not etchable like glass-based ceramics, the adhesion between resin cement and zirconia remains weak.[4] Retention of single crowns is primarily dependent on the preparation form,[5] but some restorations, such as inlay-retained or surface-retained resin-bonded FDPs, short or tapered crown preparations, or more conservative monolithic zirconia restorations, require more retention, which is usually obtained by adhesive cementation.

Several surface conditioning methods have been proposed to improve the adhesion between resin cement and zirconia.[6-14] Airborne particle abrasion with alumina particles has been used to increase the surface area and, consequently, the micromechanical retention between the zirconia surface and the resin cement.[9] Furthermore, air-abrasion with alumina particles coated by silica promotes micromechanical retention on the zirconia surface, similar to abrasion with alumina particles, with the advantage that silica is deposited on the zirconia surface.[6,15] This silica incorporated onto the zirconia surface will then be bonded to the resin cement by a silane coupling agent that acts as a link between the silica and the resin cement matrix.[11]

Since some authors have reported on the possible deleterious consequences caused by air-abrasion,[16,17] other surface conditioning methods have been proposed, such as application of an etchable glass layer onto the zirconia surface,[13] air-abrasion before zirconia sintering,[18] and etching zirconia with highly concentrated hydrofluoric acid.[19] In addition to these methods, chemical adhesion between zirconia and resin cement could be enhanced using resin cements or specific primers based on 10-methacryloxydecyl dihydrogen phosphate monomer (MDP).[20-25] In fact, bonding zirconia FDPs involves two aspects, namely zirconia-resin cement and resin cement–tooth interfaces. Consequently, it is important to employ a cementation strategy that achieves durable adhesion of resin cement to both the tooth and zirconia in order to guarantee the success of the restoration.[26] When conventional resin cements are used, conditioning the dental substrates with the corresponding adhesive resin is mandatory. In contrast, self-adhesive resin cements do not require any preconditioning of dental tissues, thereby saving clinical time. Hence, it can be anticipated that the combination of surface conditioning methods for both the tooth and zirconia and the type of resin cement affect the adhesion of zirconia.

The objective of this study, therefore, was to evaluate the effect of different adhesive cementation strategies employing different surface conditioning methods based on air-abrasion or the use of universal primer only in conjunction with conventional or self-adhesive resin cements on the adhesion of zirconia to dentin. The null hypotheses tested were that surface conditioning and resin cement type would not affect the bond strength results.

METHODS AND MATERIALS

The types, brands, manufacturers, and batch numbers of the materials used in this study are listed in Table 1.

Tooth Selection

The Committee on Ethics in Research (CEP, Process 435.230) approved this study in which molars or premolars (N=80) were used. Soft tissue and debris were cleaned from tooth surfaces using periodontal instruments and were stored in distilled water (5°C) until the experiments. Each tooth was embedded in acrylic resin (JET, Artigos Odontológicos Clássico, Ltd., São Paulo, SP, Brazil) with the aid of a metallic mold (height: 15 mm; diameter: 25 mm) and a surveyor, leaving the coronal portion exposed and the long axis of the tooth parallel to the y-axis. Enamel was removed from the occlusal surface in a cutting machine (Labcut 1010, EXTEC, Enfield, CT, USA) to expose the dentin. This surface was polished for 60 seconds with silicone carbide paper (#600) to achieve a standard smear layer. The teeth were randomly allocated into eight groups (n=10 per group) according to surface conditioning and resin cement type using a random allocation program (www.randomizer.org) (Table 2). The teeth were stored in distilled water for one week prior to cementation.

Table 1: *The Types, Brands, Manufacturers, and Batch Numbers of the Materials Used in this Study*

Material Type	Brand	Manufacturer	Batch No.
Zirconia	VITA In-Ceram YZ	Vita Zahnfabrik, Bad Säckingen, Germany	28070
Conventional resin cement (CC)	RelyX ARC	3M ESPE, St Paul, MN, USA	305960
Self-adhesive resin cement (SA)	RelyX U200	3M ESPE	473396
Alumina particles coated with silica (30 μm)	CoJet	3M ESPE	351794
Alumina particles coated with silica (110 μm)	Rocatec	3M ESPE	269078
Silane coupling agent	RelyX Ceramic Primer	3M ESPE	286040
Phosphoric acid (37%)	Condac 37	FGM, Joinvile, SC, Brazil	180612
Total-etch, single-component adhesive resin	Adper Single Bond Plus	3M ESPE	297179
Universal primer (UP)	Scotchbond Universal	3M ESPE	458640

Zirconia Specimens

Zirconia blocks (Vita InCeram YZ, Vita Zahnfabrik, Bad Säckingen, Germany) were positioned in a cutting machine and zirconia disks (diameter=4.5 mm; height=5 mm) were obtained by cutting the blocks with a cylindrical bur (diameter=4.5 mm). Zirconia disks were wet-finished using silicone carbide paper (#1200), ultrasonically cleaned (Vitasonic®, Vita Zanhfabrik, Bad Säckingen, Germany) in distilled water, and dried. The cylinders were then sintered, according to the manufacturer's instructions, in a specific oven (Vita ZYrcomat, Vita Zahnfabrik). The final dimensions of the zirconia disks after approximately 20% shrinkage were 3.4 mm in diameter and 4 mm in height. The cementation surfaces of the disks were polished with #800, #1000, and #1200 silicon carbide papers in sequence under water cooling for 60 seconds in a polishing machine (PSK-2V, Skill-tec Comércio e Manutenção Ltd, São Paulo, SP, Brazil). The disks were again ultrasonically cleaned in isopropyl alcohol for five minutes.

Cementation Procedures

Zirconia discs were conditioned according to the experimental groups (Table 2), and the dentin substrates were conditioned following the resin cement manufacturer's recommendations. The cementation surfaces of the specimens were enclosed by adhesive tape (Scotch, 3M ESPE, St Paul, MN, USA) with a hole (diameter=3.4 mm) in the middle. For the groups cemented with the conventional resin cement (CC: RelyX ARC, 3M ESPE), dentin was etched with 37% phosphoric acid for 15 seconds, washed, and gently dried with absorbent paper. One coat of adhesive resin (Adper Single Bond Plus, 3M ESPE) was then applied for 10 seconds, gently air-dried for five seconds, and photo-polymerized for 20 seconds (Radii Cal, SDI, Victoria, Australia). For the groups cemented with self-adhesive cement (SA: RelyX U200), dentin was cleaned only with water and gently dried with absorbent paper.

The base and catalyst pastes of resin cements (CC and SA) were then dispensed in equal portions, mixed, and applied to the cementation surfaces of the zirconia discs. Next, each zirconia disc was placed on its corresponding dentin, in the region defined by the adhesive tape, and a load of 750*g* was applied. Excess cement was removed, and the cementation interface was photo-polymerized for 20 seconds from four directions (Radii Cal). After polymerization, the adhesive tape was removed by cutting with a blade. One operator performed all bonding procedures (MA).

Table 2: *Abbreviation of the Experimental Groups with Respect to Surface Conditioning Methods for Zirconia and the Conditioning Parameters*

Abbreviation of the Groups	Surface Cnditioning Type for Zirconia	Conditioning Parameters
C	No conditioning—control	—
CJ	CoJet + silane	Airborne particle abrasion (duration: 4 s; pressure: 2.5 bar; distance: 10 mm) + one coat silane, waiting for 5 min
RC	Rocatec + silane	Airborne particle abrasion (duration: 4 s; pressure: 2.5 bar; distance: 10 mm) + one coat silane, waiting for 5 min
UP	Universal primer	One coat primer, rubbing for 20 s, air-dry for 5 s

Table 3: *Mean Shear Bond Strength Values (MPa), Standard Deviations, and Distribution of Frequency of Failure Types in Numbers and Percentages per Experimental Group*

Groups	Mean (±SD)	Failure Type			
		Ad-CD, No. (%)	Ad-CC, No. (%)	Mixed, No. (%)	Coh-CM, No. (%)
CC-C	5.64 ± 2.8 ^{D}	0	10 (100)	0	0
CC-RC	17.52 ± 7.4 ^{ABC}	1 (10)	5 (50)	4 (40)	0
CC-CJ	20.17 ± 6.07 ^{AB}	0	5 (50)	4	1 (10)
CC-UP	24.93 ± 7.01 ^{A}	0	4 (40)	6 (60)	0
Total CC		2.5	60	35	2.5
SA-C	10.36 ± 3.87 ^{CD}	1 (10)	6 (60)	3 (30)	0
SA-RC	16.83 ± 6.81 ^{BC}	5 (50)	1 (10)	4 (40)	0
SA-CJ	15.54 ± 4.66 ^{BC}	10 (100)	0	0	0
SA-UP	17.96 ± 5.94 ^{ABC}	4 (40)	2 (20)	4 (40)	0
Total SA		50	22.5	27.5	—

Abbreviations: Ad-CD, adhesive between cement-dentin; Ad-CC, adhesive between cement-ceramic; Coh-CM, cohesive in cement; Mixed, Coh-CM + Ad-CD or Ad-CC. For group abbreviations, see Tables 1 and 2.
[a] Different letters indicate statistical difference between the experimental groups.

Prior to the bond strength test, specimens from all groups were immersed in distilled water at 37°C and stored for 30 days.

Testing Procedure and Failure Analysis

Specimens were mounted in the jig of the Universal Testing Machine (AGS-X10kN, Shimadzu, Kyoto, Japan), and a shear force was applied to the adhesive interface until failure occurred. The specimens were positioned in the testing machine so that the adhesive interface was perpendicular to the horizontal plane, and load was applied with a knife-edge device until failure. The specimens were loaded at a crosshead speed of 1 mm/min, and the stress-strain curve was analyzed with the software program. The bond strength was calculated according to the formula $SBS = F/A$, where SBS is the shear bond strength in MPa, F was the load required for fracture (N), and A was the bonded area ($\pi x r^2$, where π=3.14 and r=1.7 mm/A=9.07 mm^2) of the specimen (mm^2).

All debonded specimens were initially evaluated under a stereomicroscope (75×) (Discovery Z-20, Zeiss, Jena, Germany). The most prevalent failure types were further observed with a scanning electron microscope (Inspect S50, FEI Company, Eindhoven, The Netherlands) (MA and FC). The types of failures were classified as follows: Ad-CD (adhesive between cement-dentin), Ad-CC (adhesive between cement-ceramic), Coh-Cer (cohesive in ceramic), Coh-D (cohesive in dentin), Coh-CM (cohesive in cement), and Mixed (cohesive in cement+Ad-CD or Ad-CC).

Statistical Analysis

Statistical analysis was performed using Statistix 8.0 software for Windows (Analytical Software Inc, Tallahassee, FL, USA). Initially, data were subjected to normality and homogeneity tests. Bond strength data (MPa) were submitted to two-way analysis of variance. Multiple comparisons were made with the Tukey post hoc test (α=0.05), with the shear bond strength as the dependent factor and surface conditioning protocols (three levels) and the resin cement types (two levels) as the independent factors. p-values of less than 0.05 were considered to be statistically significant in all tests.

RESULTS

While surface conditioning significantly affected the SBS values (p=0.0001), cement type did not (p=0.148). The interaction terms were significant (p=0.014) (Table 3).

Nonconditioned control groups presented the lowest mean SBS with both CC and SA cements. CJ and RC air-abrasion methods did not show significant difference for both CC and SA cements. The use of UP only, without air-abrasion protocols, presented mean SBS values that were not significantly different from CJ and RC conditioning methods for both cement types.

No pretest failures were observed at the end of water storage. Most failures were adhesive (Table 3). For the CC cement, the failures were predominantly Ad-CC. Conversely, for the SA cement, the failures were mainly Ad-CD. The most representative failure types are shown in Figure 1A-C.

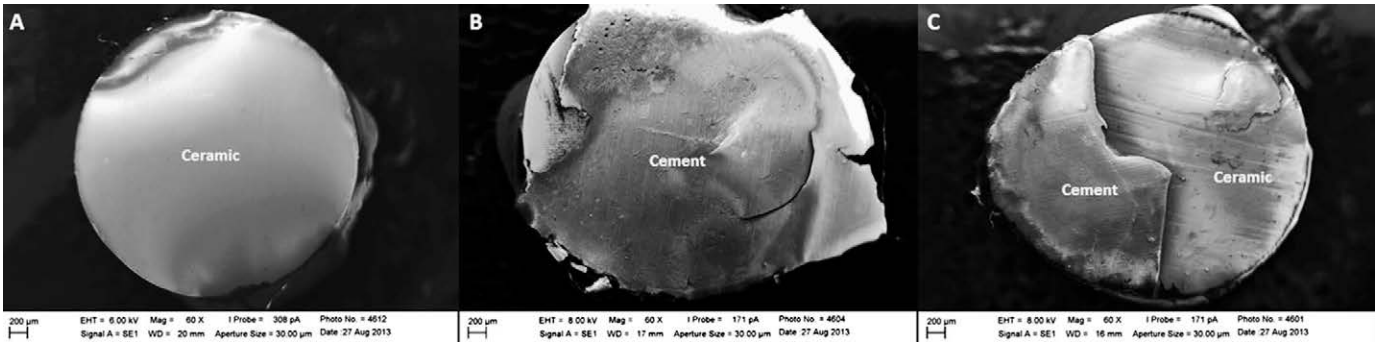

Figure 1. *(A-C) Scanning electron microscopy (SEM) micrographs (60×) of representative failure types after debonding (A) Ad-CC (adhesive between cement-ceramic), (B) Ad-CD (adhesive between cement-dentin), and (C) Mixed failure (Cohesive in cement+Ad-CD or Ad-CC).*

DISCUSSION

This study was undertaken in order to evaluate the effect of different adhesive cementation strategies employing two air-abrasion methods or the use of universal primer only in combination with conventional or self-adhesive resin cements for the adhesion of zirconia to dentin. Based on the results of this study, since surface conditioning significantly affected the adhesion results but the cement type did not, the first null hypotheses could be rejected.

It is necessary to use adhesive cementation strategies to enhance the adhesion of zirconia restorations, especially with the smaller areas available for adhering to teeth, such as inlay-retained FDPs.[25] In addition, the use of resin cements could improve the fracture resistance of all-ceramic restorations.[27] Furthermore, a ceramic restoration presents two interfaces (ceramic-resin cement and resin cement–dentin), and an adhesive-only approach can create a union between resin cement and teeth. Although it is important to measure the adhesion of ceramic restorations to the tooth substrate for better clinical significance,[9,28] most studies[13,15,22,23] to date have focused on the adhesion between zirconia and resin cement without considering the tooth aspect. In that respect, the present study could be considered more clinically relevant where the results showed that the interaction between the ceramic–resin cement–tooth interfaces is pertinent in this scenario. This was more evident not through the bond strength results but through the variations in modes of failure types.

This result corroborated with those of previous studies[29,30] in which the results showed that surface conditioning promoted higher bond strength values compared with the nonconditioned groups. For the air-abrasion surface conditioning, two particle types were used in this study (Rocatec: 110 μm, CoJet: 30 μm). Both chairside and laboratory air-abrasion versions showed similar results. This indicates that roughness did not dictate the adhesion in the resin cement–zirconia-dentin complex. With these methods, silica coating was achieved where air-abrasion with alumina coated by silica particles promoted adhesion between the silane coupling agent and the silica adhered on the zirconia surface due to the impact.[15] These results are in agreement with those of some previous studies[4,31,32] in which both particle types were compared in terms of the bond strength between resin materials and nonetchable ceramics. However, in these studies, tooth substrate was not involved. In another study[28] with similar methodology to that used in this study, both particle types presented comparable bond results. Accordingly, smaller particle size (30 μm) could be advised for air-abrasion of zirconia as this particle type would create less mechanical damage to zirconia, and chairside air-abrasion devices are certainly more cost-effective than laboratory ones.[33]

In the present study, the results expressed by the use of UP only are remarkable as a result of the fact that in these groups air-abrasion was not practiced. According to the study of Amaral and others,[23] air-particle abrasion and UP application on zirconia promoted bond strength values similar to those of the groups air-abraded with CoJet. Nevertheless, the highest bond strength values among the groups were achieved by this primer, without air-abrasion, in that study and among other primers in another study.[34]

From the clinical point of view, one single adhesive promoter for the cementation of etchable and nonetchable ceramics or other restorative materials, tooth substrate, and in intraoral repairs would eliminate multiple surface conditioning

methods.[35] In its chemical composition UP, in this case Scotchbond Universal, contains MDP, dimethacrylate, 2-hydroxyethyl methacrylate, Vitrebond copolymer, filler, ethanol, water, initiators, and silane. The mixture of these constituents could also hinder the adhesion between resin-tooth and resin-zirconia pairs, as they react differently on these two substrates.[36] Since this solution is a simplified adhesive, it contains a greater quantity of solvents.[37] Hence, if the substances could be used separately for each indication they would be more effective on each individual substrate, producing more durable results. Yet, in this study, UP was effective on the dentin-resin cement–zirconia complex.

The results of this study showed no significant effect of resin cement type. However, it has to be noted that the bond strength values obtained were related to a scenario with two interfaces. If adhesion had been measured only between resin cements and dentin, the results could have been very different as a result of the differences between the adhesion mechanisms of the two cements. The conventional resin cements require preconditioning of the tooth substrate before cementation followed by the application of the adhesive system chosen, either an etch-and-rinse (three- or two-step) or self-etch (two- or one-step) system.[37] In this study, a two-step etch-and-rinse adhesive system was used, which removes the smear layer and exposes the collagen fibers through application of phosphoric acid, followed by the application of a one-bottle adhesive.[37] After phosphoric acid etching, the surface wettability of dentin increases and better adhesion is achieved.[38] On the contrary, the self-adhesive resin cements do not require conditioning of the tooth substrate. Self-adhesive cements contain acidic monomers, which etch dentin,[39] but without phosphoric acid etching they cannot interlock micromechanically with dentin, which results in lower bond strength values.[40]

The failure types clearly indicate the differences between the effects of conventional resin and self-adhesive cements, in that, with the self-adhesive cement, adhesive failures were more commonly observed between the cement and dentin (Ad-CD). On the other hand, with conventional resin cement, adhesive failures between resin cement and zirconia (Ad-CC) were more frequent. One could conclude that the mechanism of adhesion between cement and dentin may have guided the bond strength values. However, it is important to emphasize that although the failure patterns were different, the bond values for the whole system were similar and in accordance with those reported by other studies[9,22,41] in which the self-adhesive resin cements also obtained good results.

The methodology used in this study attempted to approximate the complex clinical scenario.[28] Nevertheless, in the shear bond strength test, the manner in which the load is applied to the specimen interface typically generates an inhomogeneous stress distribution.[5] Despite this irregular distribution, when there is a "double interface" (CC and CD), in theory, the failure would initiate from the weaker interface.[11] The adhesive failure types (Ad-CD) observed in this and other studies[11] are a consequence of the weakest interface between resin cement and dentin, due to the use of self-adhesive resin cement or a conventional resin cement followed by the use of a self-etch adhesive system. Self-adhesive cements do not create favorable resin tags, but because of MDP in their composition, adhesion to zirconia substrate was enhanced. Conversely, using a microtensile bond test to study the adhesion between zirconia and resin cement with the same cements used in this study, de Castro and others[9] found no adhesive failure between cement and dentin but more cohesive failures in the cement. Hence, the adhesion between zirconia and cement seems to be stable even after some aging.[9,11] In this context, the type and duration of aging need to be critically evaluated when interpreting results.

The use of human teeth in this study could be regarded as a limitation because of differences between the ages of the collected teeth. Nonetheless, a random distribution was used to control for this factor.[42] Other aging protocols, with longer duration of water storage or thermal and mechanical cycling, should be considered in future studies in order to challenge the interfacial hydrolysis and cement degradation.[43,44] Future investigations should also verify the results of this study using other test configurations.

CONCLUSIONS

Based on this study, the following could be concluded:

1. Both chairside and laboratory air-abrasion protocols and the use of universal primer without air-abrasion improved the bond strength of zirconia to dentin with conventional and self-adhesive cements compared to nonconditioned control groups.

2. Conventional resin and self-adhesive cements showed similar mean bond strength of zirconia to dentin after 30 days of water storage.
3. While conventional resin cement presented more frequent failures between cement and zirconia, self-adhesive cement showed mainly adhesive failures between cement and dentin.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Federal University of Rio Grande do Norte (UFRN). The approval code for this study is Process n° 435.230.

Conflict of Interest

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

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Effects of Surface Treatments on the Bond Strength Between Resin Cement and a New Zirconia-reinforced Lithium Silicate Ceramic

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Clinical Relevance

Hydrofluoric acid etching promotes a high and stable resin bond to new zirconia-reinforced lithium silicate ceramic. Thus, the intaglio surface of the restorations made with this material should be etched and silanized.

SUMMARY

This study evaluated the effects of surface treatments on the bond strength between the new zirconia-reinforced lithium silicate ceramic (ZLS) and resin cement. VITA Suprinity blocks were crystallized according to the manufacturer's instructions and randomly assigned to six groups (N=36; n=6), according to the surface treatment to be performed and aging conditions: HF20, 10% hydrofluoric acid for 20 seconds, baseline (control); HF20tc, 10% hydrofluoric acid for 20 seconds, aging; HF40, 10% hydrofluoric acid for 40 seconds, baseline; HF40tc, 10% hydrofluoric acid for 40 seconds, aging; CJ, CoJet sandblasting (25 seconds, 2.5 bar, 15-mm distance), baseline; and CJtc, CoJet sandblasting (25 seconds, 2.5 bar, 15-mm distance), aging. All specimens were silanized (Monobond S) and cemented with Panavia F to newly polymerized Z250 resin blocks. After specimens were immersed for 24 hours in distilled water at 37° C, 1-mm^2 cross-section microbars were obtained by means of a cutting machine under constant cooling. Baseline groups were immediately tested, whereas "tc" groups were used to analyze the

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effect of aging on bond strength (10,000 thermal cycles, 5/55°C, 30-second bath). The microtensile bond strength test was performed with a universal testing machine (0.5 mm/min), and bond strength (MPa) was calculated when the load-to-failure (N) was divided by the adhesive area (mm^2). We also evaluated the surface roughness (Sa, average roughness; Str, texture aspect ratio; Sdr, developed interfacial area ratio) and the contact angle resulting from the treatments. Data were statistically analyzed by one- or two-way analysis of variance and Tukey's test (all α=5%). The failure mode of each specimen was evaluated by stereomicroscopy, and representative specimens were analyzed by scanning electron microscopy. The microtensile bond strength was affected by the surface conditioning ($p<0.0001$), storage condition ($p<0.0001$), and the interaction between them ($p=0.0012$). The adhesion for HF etching was stable, whereas for CJ, aging significantly damaged the adhesion. Most failures were predominantly adhesive between ceramic and cement (52.6%). The roughness of the treated samples was higher compared with that of polished specimens for the three evaluated parameters (Sa, Str, and Sdr; all $p<0.0001$). Contact angle was also influenced by treatments ($p<0.0001$), with the CJ group showing values similar to those of control specimens. It can be concluded that the three surface treatment techniques present favorable immediate results, but silica coating was not effective in maintaining the bond strength over the long term.

INTRODUCTION

Glass ceramics have evolved over the years in their compositions and processing techniques. Leucite or lithium disilicate were the main crystal phase of the first generation of these materials, which were marketed in ingots for injection. Nowadays, the glass ceramics are mainly lithium disilicate–based pressable ingots or computer-aided design and computer-aided manufacturing (CAD-CAM) blocks. These restorations have satisfactorily served as monolithic restorations.[1] Recently, a new material, zirconia-reinforced lithium silicate ceramic (ZLS), was launched under the argument that zirconia could act as a crystal phase that can reinforce the material; that is, avoid crack propagation.

The ZLS represents an attempt to unite resistance properties of polycrystalline ceramics with the esthetic excellence of the glass-ceramics in a monolithic restoration. Moreover, because a ceramic matrix is predominantly glass (from 8% to 12% zirconia), this material is considered acid sensitive and susceptible to hydrofluoric acid etching, unlike polycrystalline ceramics.[2]

The classification system proposed by Valandro and others[2] was based on the existence of ceramic surface degradation by hydrofluoric acid (HF). Ceramics with high glass content in their composition, such as feldspar-, leucite-, and lithium disilicate–based ceramics, suffer as a result of the action of HF, resulting in a micromechanical retentive surface and thus are called acid sensitive. Ceramics based on glass infiltrated alumina or zirconia, densely sintered alumina, and yttria-tetragonal zirconia polycrystal (Y-TZP) do not degrade with HF, do not present micromechanical retention and undercuts,[2] and are referred to as acid resistant.

The extension of the etching time indicated by the manufacturer for an acid-sensitive ceramic was analyzed by Zogheib and others,[3] who concluded that lithium disilicate ceramic requires more than 60 seconds of HF etching for the creation of effective microretention.[3] Menees and others[4] found that HF etching for 20 seconds in concentrations varying from 5% and 9.5% is enough to remove the glass matrix. Despite extensive removal for 120 seconds, it was clear that the resulting etch pattern for these conditions was uniform and was not enough to affect the bending strength of the lithium disilicate ceramic.[4]

However, the use of HF requires careful attention due to its potential risk for the degradation of organic matter,[5-7] and for this reason, other options have been investigated for ceramic surface treatment, including air abrasion with silica-coated aluminum oxide particles. During silica coating, the high energy of the shock resulting from the aluminum oxide particles is responsible for the fusion of these silica particles to the ceramic surface, making it chemically reactive to the resin cement through the silane agent and also increasing the bond strength to ceramics.[8,9]

Conversely, silica coating treatment is controversial, because some authors reported a decrease in mechanical strength of the material[10-12] and the induction of crack propagation,[13] whereas others have shown no deleterious effect on long-term mechanical behavior.[11-18] There are no data in the literature on the effects of silica coating on lithium silicate reinforced by zirconia ceramic. We believe this type of surface treatment can be particularly

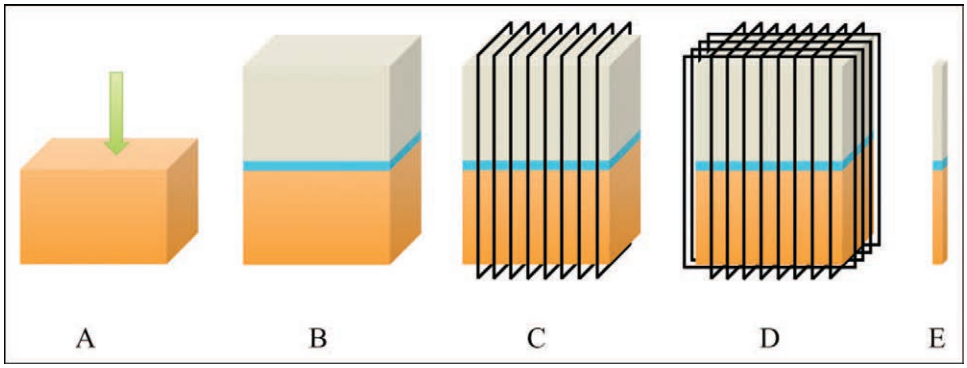


Figure 1. *Sketch of the sample fabrication process for microtensile bond strength test. Orange = ZLS ceramic; blue = resin cement; beige = Composite. (A): Surface treatments (HF20, HF40, or CJ) were performed on ZLS blocks that were then cemented with a resin cement to newly polymerized composite resin blocks (B). Twenty-four hours after polymerization, blocks were cut into x (C) and y (D) axes. One half of the specimens of each treatment were thermo cycled (HF20tc, HF40tc, and CJtc; n=6), whereas the other half were immediately tested (HF20, HF40, and CJ; n=6). In total, 120 specimens per group were tested using the microtensile bond strength test.*

important if the zirconia content present in in ZLS makes it less sensitive to acid conditioning.

The maintenance of bond strength in the long term is a factor that must be considered, because studies have shown that thermocycling decreases bond strength between the resin cement and glass-ceramic.[9]

The aim of this study was to evaluate the effects of surface treatments on the bond strength of a new ceramic lithium silicate reinforced by zirconia and resin cement. The hypothesis that extended etching time and silica coating result in stronger and longer-lasting bond strength was tested.

METHODS AND MATERIALS

The ZLS ceramic (Vita Suprinity, Vita Zahnfabrik, Bad Säckingen, Germany) was cut into blocks (12 × 8 × 7 mm) and crystallized according to the manufacturer's instructions. Blocks were embedded in acrylic resin bases, and the top face of each ceramic was polished with 800-grit sandpaper granulation under constant cooling in a polishing machine.

Ceramic Surface Treatment

The blocks (N=36) were randomly divided into six groups (n=6), and these were subjected to six experimental conditions established by the two factors under study: treatments (at three levels: HF20, HF40, and Cojet) and aging (at two levels: absence and presence).

For this, ZLS blocks were treated according to surface treatments and aging conditions: HF20, etching with 10% hydrofluoric acid (Condacporcelana, FGM, Joinville, Brazil) for 20 seconds, followed by rinsing for the same time (control protocol indicated by the manufacturer[19]); HF20tc, HF20 treatment + aging; HF40, etching with 10% hydrofluoric acid (Condacporcelana, FGM) for 40 seconds, followed by washing for the same time; HF40tc, HF40 treatment + aging; CJ, air-abrasion with 30 micron silica-coated alumina particles (CoJet Sand, 3M ESPE, Seefeld, Germany; 25 seconds, 2.5 bar, 15-mm distance); and CJtc, CJ treatment + aging. All specimens were silanized (Monobond S, Ivoclar Vivadent, Schaan, Liechtenstein) and cemented, one at a time, with a resin cement (Panavia F, Kuraray, Okayama, Japan) to composite resin (Filtek Z250, 3M ESPE, Irvine, CA, USA) blocks that had been made and polymerized just before cementation. Thus, six repetitions per group were obtained. Figure 1 presents a chart of specimen preparation.

Microtensile Bond Test

After 24 hours in distilled water at 37°C, 1-mm^2 cross-section microbars composed of ceramic/cement/resin were obtained by means of a cutting machine (ISOMET 1000, Buehler, Lake Bluff, IL, USA) under constant cooling. The ends of the blocks were demarcated, and microbars from this area were excluded. The microtensile bond strength of all microbars from the same block was averaged to obtain the mean value for that block. Twenty microbars were randomly selected from each block and included in the analysis.

Microbars obtained from blocks of the baseline groups were immediately tested. Microbars from the "tc" groups were used to analyze the effect of aging on bond strength. For this, they were subjected to 10,000 thermal cycles in water at a temperature ranging between 5°C and 55°C, with 30 seconds of immersion and five seconds of transition (bath for the 521-6D cycle test, Ethik Technology, Vargem Grande, São Paulo, Brazil).

Table 1: *Means (MPa), Standard Deviations (SD), and Homogeneous Groups by Tukey's Test (α=5%)*

Groups	Mean (SD)
HF20	32.33 (4.1)[a]
HF20tc	32.20 (4.9)[a]
HF40	32.07 (6.7)[a]
HF40tc	22.71 (1.9)[a]
CJ	24.46 (4.1)[a]
CJtc	5.62 (8.9)[b]

Means that do not share a letter are significantly different. Abbreviations: CJ, CoJet air-abrasion (25 seconds, 2.5 bar, 15-mm distance); and CJtc, CJ treatment + aging; HF20, etching with 10% hydrofluoric acid for 20 seconds, followed by rinsing for the same time (control protocol indicated by the manufacturer[19]); HF20tc, HF20 treatment + aging; HF40, etching with 10% hydrofluoric acid for 40 seconds, followed by washing for the same time; HF40tc, HF40 treatment + aging.

After specimen dimensions were measured with a digital caliper, specimens were attached to the testing device (OG01, Odeme, Lucerne, Brazil) with cyanoacrylate (superglue, Loctite, Lucerne, Brazil). The microtensile bond strength test was performed in a universal testing machine (DL-1000, EMIC, São José dos Pinhais, Brazil; 0.5 mm/min), and bond strength (MPa) was calculated by dividing the load-to-failure (N) by the adhesive area (mm^2). A mean value for each block was calculated and used in the data analysis.

Failure Analysis

The fractured specimens were examined by stereomicroscopy (Stereo Discovery V20, Zeiss, Göttingen, Germany), and failure mode was classified as resin or resin cement cohesive, predominantly adhesive between resin cement and ceramic, mixed, or ceramic cohesive.

Roughness and Topographic Analysis

For additional analysis of the effects of surface treatments on ZLS ceramic, we obtained 2 × 1 × 1-mm specimens following the same procedures as used in the ceramic blocks for microtensile testing (cutting, polishing, and surface treatments). One sample was used as a control group and received only polishing.

Table 2: *Two-way Analysis of Variance for Microtensile Bond Strength Data*

Source	SS	df	Ms	*F*	*p* value [a]
Treatments	1890.0	2	945.1	30.65	<0.0001
Aging	801.6	1	801.6	25.99	<0.0001
Interaction	524.8	2	262.4	8.51	**0.0012**
Error	925.1	30	30.8		
Total	4142	35			

[a] :*p value in bold indicates significant difference in Microtensile Bond Strength values (p<0.05). Abbreviations: SS: sum of squares; df: degrees of freedom; MS: mean square.*

Table 3: *Data (Number and Percentage) of Tested Samples and Pre-test Failures*

Groups	Number of possible specimens	Pretest failure during cut, %	Number of specimens obtained	Pretest failure, %
HF20	120	2 (1.6)	118	0
HF20tc	120	4 (3.2)	116	0
HF40	120	6 (5)	114	0
HF40tc	120	2 (1.6)	118	0
CJ	120	0 (0)	120	0
CJtc	120	1 (0.8)	119	114 (95.8)
Total	720	15 (2%)	705	114 (16.17%)

Abbreviations: CJ, CoJet air-abrasion (25 seconds, 2.5 bar, 15-mm distance); and CJtc, CJ treatment + aging; HF20, etching with 10% hydrofluoric acid for 20 seconds, followed by rinsing for the same time (control protocol indicated by the manufacturer[19]); HF20tc, HF20 treatment + aging; HF40, etching with 10% hydrofluoric acid for 40 seconds, followed by washing for the same time; HF40tc, HF40 treatment + aging.

The roughness of the specimens was evaluated in five samples of each group by means of a digital optical profiler (Wyko NT 1100, Veeco, Plainview, NY, USA), connected to the Wyko Vision 32 (Wyko, Veeco) at 20× magnification and 301.3 × 229.2 μm of analysis area. We obtained values (nm) for height (Sa, average roughness), spatial (Str, texture aspect ratio), and hybrid (Sdr, developed interfacial area ratio) parameters.

The contact angles of treated surfaces were also evaluated in six samples from each group before and after silanization by means of an optical tensiometer (TL 1000, Theta Lite Attention, Lichfield, Staffordshire, United Kingdom) by the sessile drop technique. For this, a syringe (#1001 Gastight Syringes, 1 mL, Hamilton, Reno, NV, USA) deposited a drop of distilled water on the sample surface. After 10 seconds of waiting for the drop to settle, a series of 30 images per second was recorded by the equipment for 20 seconds. OneAttension (Biolin Scientific, Lichfield, Staffordshire, United Kingdom) software was used for calculation of the average values of contact angle for each sample from the images obtained.

Finally, the morphology of the surface was analyzed by scanning electron microscopy. For this, the sample surface was coated with a thin layer of gold in low atmospheric pressure by means of an ion sputter-coater (SC7620 'Mini' Sputter Coater/Glow Discharge System, Emitech, East Sussex, United

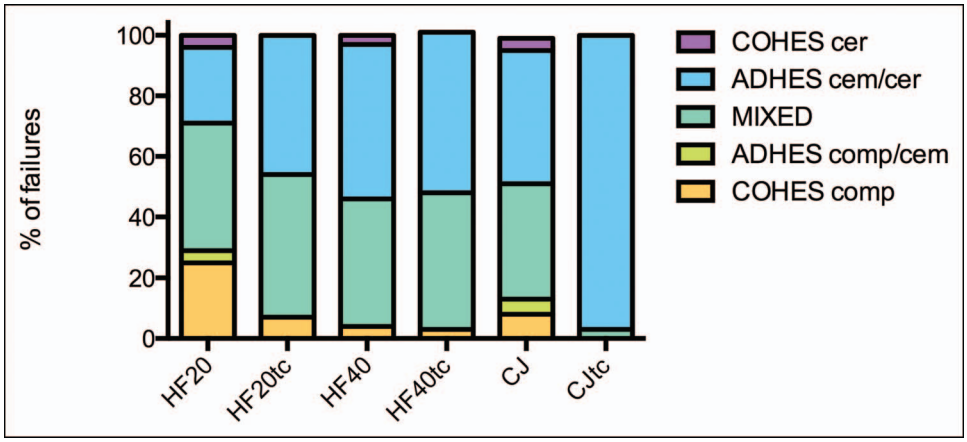


Figure 2. *Bar graph of failure type distribution. ADHES cem/cer = predominantly adhesive failure between resin cement and ceramic; ADHES comp/cem = predominantly adhesive failure between composite resin and cement; COHES cer = cohesive failure of ceramic; COHES comp = cohesive failure of composite/resin cement; MIXED = cohesive and adhesive failures.*

Kingdom), and the topography was analyzed and photographed with high-vacuum equipment (Inspect S 50, FEI Company, Brno, Czech Republic) operating at 20-25 kV, 5.0 spot, and magnifications of 500× and 5000×.

Data Analysis

Data were tabulated, and the blocks were used as experimental units for statistical analysis of the microtensile bond strength data. The results of bond strength were analyzed by two-way analysis of variance (ANOVA) (treatment and aging) and Tukey's test (both α=5%), and roughness and contact angle values were analyzed by one-way ANOVA and Tukey's test (both α=5%).

RESULTS

The microtensile bond strength results are presented in Table 1. ANOVA showed that interaction of surface and aging influenced the bond strength (p=0.0012) (Table 2). The adhesion for the HF groups was stable, whereas for CJ, aging significantly damaged the adhesion. Samples in the CJtc group also showed a large number of pretest failures after thermal cycling (Table 3).

Failure was predominantly adhesive between the ceramic and resin cement (52.6%) (Figure 2).

Surface roughness was also influenced by surface treatments for all parameters evaluated: Sa (p<0.0001), Str (p<0.0001), and Sdr (p<0.0001) (Figure 3). All treatments increased roughness parameters compared with those of control samples (polished, without treatment).

The contact angle was also influenced by surface treatments for both silanized (p<0.0001) and unsilanized samples (p<0.0001). In both cases, the group conditioned with hydrofluoric acid for 40

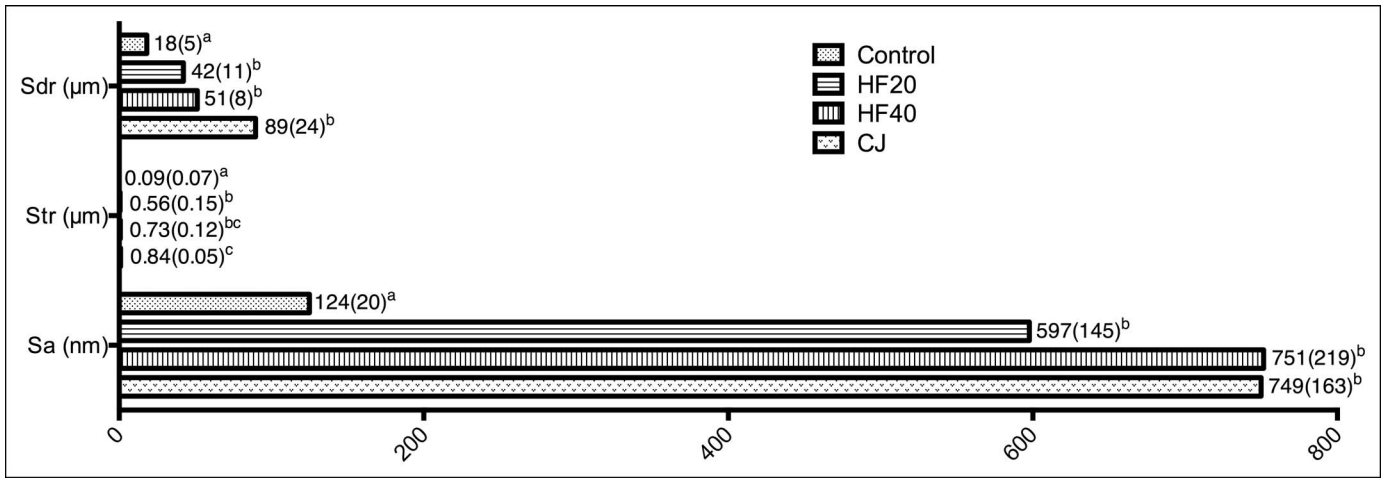
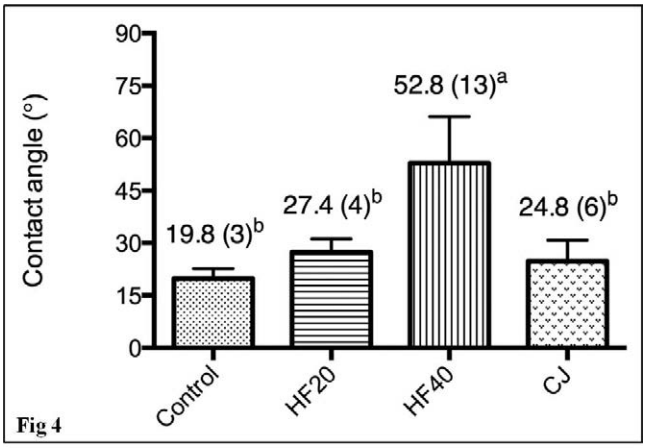


Figure 3. *Bar graph of roughness values for tested groups. For each roughness parameter, means with different superscript letters are significantly different.*



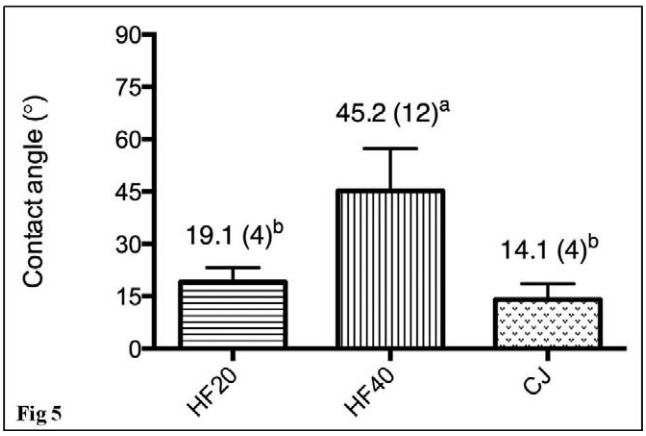


Figure 4. *Bar graph of the mean values of contact angle (°) for tested groups (not silanized). Means with different superscript letters are significantly different.*

Figure 5. *Bar graph of the mean values of contact angle (°) for silanized specimens. Means with different superscript letters are significantly different.*

seconds (HF40) showed the highest wettability values (Figures 4 and 5).

Figure 6 shows representative profilometry images and scanning electron microscope micrographs of the surface patterns for tested groups.

DISCUSSION

The baseline assessment showed that the three treatments tested (HF20, HF40, and CJ) had similar baseline bond strength values.

In the context of surface treatment by extension of the etching time suggested by the manufacturer, studies have found no deleterious effect of hydrofluoric acid on the resistance of glass-ceramic,[20,21] because the excessive etching reduced existing surface faults in size and depth, besides removing or stabilizing surface defects.[22,23] Thus, increasing the etching time can enhance the materials strength[24] but does not lead to improved bond strengths, according to our findings.

We found that the thermocycling had a negative effect on the bond strength, and samples in the silica-coated group (CJtc) showed an almost 80% decrease in the microtensile bond strength to resin cement. Most specimens in this group did not resist the thermal cycling, as well as the large percentage of predominantly adhesive failures between resin cement and ceramic showed that the surface modification by the addition of silica did not guarantee a stable bonding between ZLS and resin cement in the long term. According to Kern and Thompson,[25] this is attributable to the fact that the irregularities created by the sandblasting are devoid of microretention. Corroborating this, Menees and others[4] argued that for the ceramic lithium disilicate ceramic, hydrofluoric acid etching introduces more uniform and better-distributed surface changes.

In fact, silica-coated topography in our study confirms the existence of small irregularities, although the surface roughness of the three groups were not much different. In this same context, Kern and Wegner[12] stated that, because of this limited ceramic roughness, chemical and physical Bis-GMA (Bisphenol A-glycidyl methacrylate)–based resin bonds are not water resistant and end up suffering detachment.

For roughness parameters, the three treatments were effective in changing the surface pattern, generating similar results. When a glass-ceramic surface is exposed to HF etching, a selective removal of its vitreous matrix occurs, exposing the crystalline structure[6,26] and resulting in a topography with a total contact area greater than that of a smooth surface.[27] Silica deposition generates an irregular rough surface with increased surface area that improves ceramic wettability. However, sandblasting can also induce excessive gaps in the surface of the material or even a significant loss of material.[25,28]

This conformation of the material's surface topography is closely linked to the characteristics that it presents in terms of contact angle and consequent wettability.[29]

The contact angle measurement of the dispersion liquid (often water of high purity) on a substrate is used as an indicator of total surface energy and wettability of the substrate.[27,30] The smallest contact angle resulting from silica coating represents better surface wettability. However, the silica coating

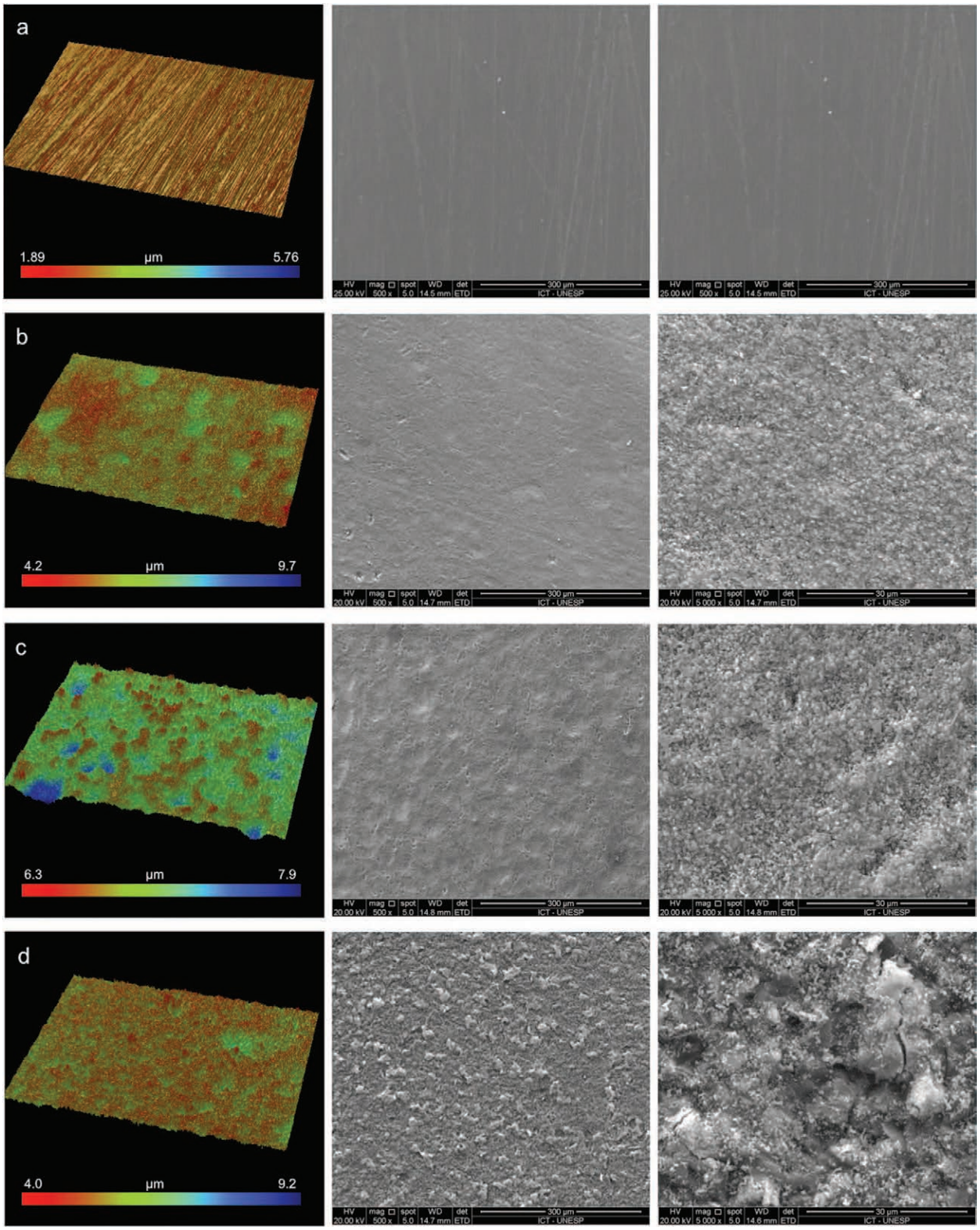


Figure 6. *Representative images of surface patterns of the tested groups. Left column: profilometry images. Center column: micrographs, 500×. Right column: micrographs, 5000×. Line A: control samples (only polished). Line B: HF20. Line C: HF40. Line D: CJ.*

showed a reduction in bond strength after aging, suggesting that, although this technique ensured good initial bond strength to resin cement, the pattern generated by silica coating is not favorable for the long-term maintenance of this resistance.[29,31] The lower bond strength obtained with acid etching for 40 seconds is probably due to the removal of a greater quantity of glass matrix and exposure of lithium silicate crystals and particles of zirconia creating a surface with lower wettability.

A few microbars were lost during cutting. Most microbars showed predominantly adhesive failure between ceramic and resin cement. Thus, despite the decline in bond strength of the HF40 group, the CJ group was most vulnerable to temperature changes in the water, with great loss of microbars during thermocycling.

Therefore, considering the results of microtensile bond strength, surface roughness, contact angle, and morphology obtained for surface treatments and considering the potential risks with the use of HF, it is suggested that one should not extend the time suggested by the manufacturer. The use of silica coating is also not advantageous for long-term maintenance of the bond.

CONCLUSION

On the basis of our results, it can be concluded that the silica coating was not efficient in maintaining the bond strength after aging, and etching with hydrofluoric acid for 20 or 40 seconds was equally effective in producing stable resin bonding to a ZLS ceramic.

Conflict of Interest

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

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Influence of Restoration Type on the Cytotoxicity of a 35% Hydrogen Peroxide Bleaching Gel

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Clinical Relevance

The application of a 35%-H_2O_2 bleaching gel to a dental surface containing a hydrolytically degraded resin-modified glass ionomer cement restoration results in more intense diffusion of H_2O_2 into the pulp chamber, which increases pulp cell cytotoxicity.

SUMMARY

Objectives: The tooth/restoration interface may act as a pathway for hydrogen peroxide (H_2O_2) diffusion into the pulp chamber. Therefore, the influence of resin-modified glass ionomer cement (RMGIC) and resin composite simulated restorations on the cytotoxicity of an in-office bleaching gel was assessed *in vitro*.

Materials and Methods: Cavities in enamel/dentin discs restored with RMGIC Vitremer (3M ESPE) or Single Bond/Filtek Z350 (3M ESPE) resin composite (RC) were subjected or not subjected to hydrolytic degradation (HD). A 35%-H_2O_2 bleaching gel was applied to simulated restored and nonrestored enamel surfaces, and culture medium in contact with the dentin substrate (extract) was collected and applied to MDPC-23 cells. Nonrestored discs subjected or not subjected to bleaching were used as positive and negative controls, respectively. Cell viability, oxidative stress, interleukin (IL)-1β expression, alkaline phosphatase (ALP) activity, and mineralized nodule deposition were evaluated. The H_2O_2 in the extracts was quantified. Data were subjected to statistical analysis.

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Results: Higher oxidative stress associated with reduced cell viability, ALP activity, and mineralized nodule deposition was observed for all bleached groups compared with the negative control group. The RMGIC/HD group, which presented the highest H_2O_2 diffusion, had the lowest values of cell viability, ALP activity, and mineralized nodule deposition, as well as significantly increased IL-1β expression.

Conclusions: Dental cavities restored with the RMGIC subjected to hydrolytic degradation allowed for more intense diffusion of H_2O_2 into the pulp chamber, intensifying the toxicity of a 35%-H_2O_2 bleaching gel to pulp cells.

INTRODUCTION

Vital tooth bleaching is a cosmetic technique widely used in clinical dental practice. However, this procedure is characterized by a high prevalence of tooth sensitivity, which may cause considerable to intolerable discomfort to patients.[1-5] This side effect has been associated with the rapid transtooth diffusion of hydrogen peroxide (H_2O_2),[6,7] which is toxic to pulp cells.[8-12] An important factor that can influence the diffusion of H_2O_2 into the pulpal space is the presence of cavity restorations on tooth surfaces.[13-15] It has been shown that the tooth/restoration interface may act as a pathway for H_2O_2 diffusion into deep dentin and, consequently, into the pulp chamber, depending on the dental material used to create the restoration.[14,15] Bonafé and others[16] showed that the application of a 35%-H_2O_2 bleaching gel to anterior teeth containing aged resin composite restorations resulted in more intense and prevalent tooth sensitivity. The authors reported that the dental material and technique used to restore dental cavities, as well as the quality of restoration margins, may affect H_2O_2 diffusion. This fact was corroborated by Soares and others,[17] who showed that resin composite (RC) restorations performed with etch-and-rinse adhesives did not influence the transtooth cytotoxicity of a professional bleaching protocol. However, a significantly higher toxic effect was observed when a two-step self-etch adhesive was used to perform such adhesive restorations.[18]

Instead of the limited range of clinical indications, resin-modified glass ionomer cements (RMGICs) have been used in operative dentistry to restore carious or noncarious cervical lesions in permanent teeth, since these areas are not subjected to compressive loads.[19] The main advantage of this kind of dental material in this clinical situation is related to the chemical adhesion to tooth structure, biocompatibility, and release of fluoride.[19] However, the bond strength of RMGICs to enamel and dentin is lower than that observed for RC restorations,[20,21] making the application of bleaching gels to tooth/RMGIC restorative material interfaces a concern.[22] Therefore, the present study evaluated the effects of RMGIC and etch-and-rinse adhesive/RC restorations, subjected or not subjected to hydrolytic degradation, on the indirect toxicity of a 35%-H_2O_2 bleaching gel to odontoblast-like cells. The null hypothesis is that these restorations have no effect on the cytotoxicity of the bleaching protocol.

METHODS AND MATERIALS

Specimen Preparation

Standardized 3.5-mm-thick and 5.6-mm-diameter enamel/dentin discs were obtained from intact bovine incisors, as previously described.[12] Round cavities measuring 1.6 mm in diameter and 2.5 mm deep were prepared on part of each disc by means of a high-speed, water-cooled cylindrical diamond bur (#1095; KG Sorensen, Barueri, SP, Brazil). Therefore, the remaining dentin thickness between the base of the cavity preparation and the simulated pulp chamber wall was set to 1.0 mm.[17] The cavities were restored with a RMGIC or with RC associated with two-step etch-and-rinse adhesive, following the manufacturer's instructions, as described in Table 1. The etch-and-rinse adhesive/RC restoration was used as a control since it was previously demonstrated that this restoration does not impact the transenamel and transdentinal MDPC-23 cell cytotoxicity when bleaching, even when it is subjected to hydrolytic degradation (HD).[17]

In the nonrestored discs, no cavity preparation was performed; however, a resin coating was prepared on the enamel surface, with the same diameter as that of the restoration performed on discs of restored groups. Therefore, the enamel surface subjected to bleaching was standardized in both restored and nonrestored groups. For the nonrestored groups, a round enamel surface with 1.6-mm diameter was etched with 37% phosphoric acid for 30 seconds, followed by the application of two layers of bonding agent and light-curing for 20 seconds. Finally, a layer of composite resin (1.0 mm thick × 1.6 mm diameter) was applied and also light-cured for 20 seconds (Figure 1A).

Table 1: *Application Protocol for Each Restorative Material*

Material	Application Protocol
RMGIC (Vitremer; 3M ESPE, St Paul, MN, USA)	A brush was used to apply the Vitremer primer (HEMA+polycarboxylic acid) for 30 seconds to enamel and dentin, followed by gentle air-drying for 15 seconds. This process was repeated once more, and the primer was then light-cured (450 mW/cm^2 Curing Light XL 300, 3M ESPE) for 20 seconds.
	One level scoop and one drop of liquid of Vitremer were dispensed onto the mixing pad and mixed within 45 seconds.
	The material was applied to the cavity by means of a Centrix applicator and light-cured for 40 seconds.
	Twenty-four hours after cavity restoration, the restoration surface was polished with sequential Soflex discs (3M ESPE) at low speed.
RC (Single Bond + Filtek Z350; 3M ESPE, St Paul, MN, USA)	The 37% phosphoric acid etchant (Scotchbond, 3M ESPE) was applied to enamel for 30 seconds and to dentin for 15 seconds and then rinsed for 30 seconds, and the tooth structure was dried with sterilized cotton.
	The first layer of Single Bond was applied under friction, followed by 30 seconds of resting and 10 seconds of gentle air-drying. A second bonding agent layer was applied, and the product was light-cured for 20 seconds under a halogen lamp (450 mW/cm^2 Curing Light XL 300, 3M ESPE).
	The cavity was restored by the application of two increments of the nanofilled composite resin Filtek Z350, which were individually light-cured for 20 seconds.
	Twenty-four hours after cavity restoration, the resin surface was polished with sequential Soflex discs (3M ESPE) at low speed.

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; RC, resin composite; RMGIC, resin-modified glass ionomer cement.

Half of the restored discs were subjected to HD, which consisted of a thermocycling regimen in a thermal cycler (MSCT-3 plus; Marcelo Nucci-ME, São Carlos, SP, Brazil) for a total of 20,000 cycles at 5°C and 55°C, with a 30-second dwell time in each bath, followed by 12 months of storage at 37°C in 0.1% thymol-buffered solution, which was replaced weekly. The thymol solution was used to prevent microbial contamination during the storage periods. The dentin surfaces of discs were treated with ethylenediaminetetraacetic acid (EDTA), 0.5 N, for 30 seconds for smear layer removal, and discs were then individually adapted to artificial pulp chambers (APCs) (Figure 1B) by means of two silicone O-rings (Rodimar Rolamentos Ltda, Araraquara, SP, Brazil). To avoid any H_2O_2 diffusion through the edges of the discs, a seal was created with wax on the silicone ring area. The APCs with the enamel/dentin discs in position were sterilized in ethylene oxide gas.

Experimental Procedure

The disc/APC sets were positioned in 24-well plates (Costar Corp, Cambridge, MA, USA) containing 1 mL of serum-free Dulbecco Modified Eagle Medium (DMEM; Sigma-Aldrich, St Louis, MO, USA). A 35% H_2O_2 bleaching gel (Whiteness HP; FGM Produtos Odontológicos Ltda, Joinville, SC, Brazil) was applied to simulated restoration and enamel surfaces of the discs three times for 15 minutes each time. The groups are described in Table 2. Two control groups were used in the present investigation. Nonrestored/nonbleached discs were used in the negative control group (NC), which represents normal cell parameters. The nonrestored/bleached discs were used in the positive control group (PC), which represents the cell responses to bleaching. In total, six APC/disc sets were used for each experimental group. The MDPC-23 cells were seeded on 96-well plates (Costar Corp) at 80% confluence (6000 cells/well; 48 hours) in

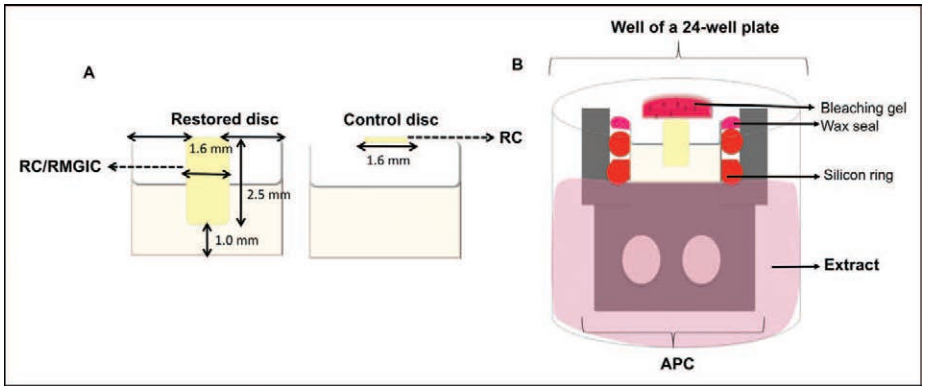

Figure 1. *Representative illustration of experimental protocol. (A) Restored and control discs. (B) APC/disc set positioned in a well of a 24-well plate.*

Table 2: *ACP Disc/Set Distribution into Experimental and Control Groups*

Group	Treatment	Restorative Material	Aging Procedure
NC	Nonbleached	Nonrestored	Water storage – 24 h
PC	Bleached	Nonrestored	Water storage – 24 h
RMGIC	Bleached	RMGIC	Water storage – 24 h
RMGIC/HD	Bleached	RMGIC	Water storage – 12 mo + thermocycling
RC	Bleached	RC	Water storage – 24 h
RC/HD	Bleached	RC	Water storage – 12 mo + thermocycling

Abbreviations: HD, hydrolytic degradation; NC, negative control group; PC, positive control group; RC, resin composite; RMGIC, resin-modified glass ionomer cement.

DMEM plus 10% fetal bovine serum (FBS; Sigma-Aldrich). Immediately after bleaching, the culture medium in contact with the internal dentin surface (extract) of each APC/disc set was collected and distributed into aliquots of 100 μL, which were applied to the cultured cells. The MPDC-23 cells were incubated with the extracts for a period of 1 hour at 37°C and at 5% CO_2, and then the experimental protocols were conducted, as follows.

Cell Viability

For cell viability analysis, the methyl tetrazolium (MTT) assay was performed. Immediately after the one-hour contact time with extracts, the cells were incubated for four hours with MTT solution (5 mg/mL; Sigma-Aldrich) diluted in DMEM without FBS (1:10), and the absorbance of formazan crystals on viable cells was read at a 570-nm wavelength (Synergy H1, Biotek, Winooski, VT, USA). The numeric values obtained from the MTT assay were converted into percentages according to mean absorbance observed in the NC, which was considered as 100% of cell viability.

Interluekin (IL)-1β Protein Expression

The enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA) was used for this analysis. Two 100-μL aliquots of the extracts from each APC/disc set were applied to two wells (100 μL per well) with previously seeded MDPC-23 cells. Immediately after the contact time with extracts, the cells were incubated for 24 hours in DMEM with no FBS (100 μL). The culture media of the two wells of each APC/disc set were then collected (total of 200 μL) and incubated for two hours in ELISA plates previously coated with the primary antibody (overnight at room temperature) and blocked with bovine serum albumin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) solution (one hour). Secondary antibody was then added, followed by reagent and stop solutions. Protein expression was analyzed by spectrophotometry (455 nm) with standard curves containing defined concentrations of the cytokine (Synergy H1, Biotek).

Oxidative Stress Generation

The production of reactive oxygen species (ROS) in cultured cells was quantified by means of a cell-permeant fluorescence probe 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA; Life Technologies, San Francisco, CA, USA) (n=6). The cells were incubated at 37°C and at 5% CO_2 with 5 μM carboxy-H_2DCFDA for 10 minutes and were then exposed for one hour to the extracts (100 μL) of each experimental group. After this period, the fluorescence intensity was monitored at 592-nm excitation and 517-nm emission by means of a microplate fluorescence reader (Synergy H1, Biotek).

Alkaline Phosphatase (ALP) Activity

Immediately after the contact with the extracts, the cells were cultured in osteogenic medium (DMEM plus 10% FBS, supplemented with 10 nmol/L β-glycerophosphate and 50 μg/mL sodium ascorbate; Sigma Chemical Co, St Louis, MO, USA) for seven days (the medium was changed daily). After the seven-day incubation period, the ALP activity was assessed (n=6). Cell lysis was performed (0.1% sodium lauryl sulfate; Sigma), and an aliquot was transferred to glass tubes containing thymolphthalein monophosphate substrate at 37°C (End point assay; Labtest, Lagoa Santa, MG, Brazil). After a 10-minute incubation period at 37°C, the color reagent (sodium carbonate and sodium hydroxide) was added, and the absorbance was read at a 590-nm wavelength (Synergy H1, Biotek), which was converted into U/L by means of a standard curve. Total protein (TP) dose was performed for normalization of ALP according to the Read and Northcote protocol, as previously described.[10] The absorbance of the test and blank tubes was measured at a 655-nm wavelength (Synergy H1, Biotek) and converted into mg/L by a standard protein curve. The final value of ALP was normalized by TP obtained from each well,

with the value of ALP activity measured as U/mg of protein. The mean ALP activity value of the NC was considered as 100% of ALP activity, and the percentage value for each sample was calculated based on this parameter and used for statistical analysis.

Mineralized Nodule Deposition

For assessment of the quantity of mineralized nodules deposited (n=6), Alizarin Red staining was performed. The cell cultures after the incubation period with extracts were also cultured in osteogenic medium for seven days. The cells were then fixed with cold 70% ethanol for one hour, washed with deionized water, and then stained with Alizarin Red dye (40 mM, pH 4.2; Sigma-Aldrich) for 20 minutes under gentle shaking (VDR Shaker, Biomixer, Ribeirão Preto, SP, Brazil). After aspiration of unincorporated dye, the cells were washed twice with deionized water for the removal of excess stain. The cells were then incubated with 10% cetylpyridinium chloride (Sigma-Aldrich) for 15 minutes under agitation to solubilize the nodules. The absorbance of the resulting solution was measured at 570 nm (Synergy H1, Biotek). The percentage of calcium deposition for each experimental group was calculated based on the mean value of the NC as 100% of staining.

Quantification of H_2O_2 Diffusion

The amount of H_2O_2 present in the extract was also quantified. Aliquots of 100 μL obtained from extracts of each group were transferred to tubes containing 900 μL of acetate buffer solution (2 mol/L, pH 4.5) to stabilize the H_2O_2. After that, an aliquot of 500 μL of the buffer solution plus H_2O_2 from each tube was transferred to test tubes containing 2.2 mL of deionized water and 250 μL of 0.5 mg/mL leucocrystal violet (Sigma-Aldrich Corp). The tubes were agitated, and a 50-μL quantity of horseradish peroxidase enzyme solution (1 mg/mL; Sigma-Aldrich Corp) was added. Then, aliquots of each tube were transferred to 96-well plates, and the optical density of the solutions was measured at 600-nm wavelength (Synergy H1, Biotek). A standard curve of known H_2O_2 concentrations was used for conversion of the optical density obtained in the samples into μg of H_2O_2 per mL of extract.

Statistical Analysis

To verify the reproducibility of data, we performed two independent experiments for all protocols in this study. Thereafter, data were compiled and subjected to the Levene test for the verification of homoscedasticity. Data from cell viability, H_2O_2 diffusion, Alizarin Red, and ALP activity were subjected to one-way analysis of variance and Tukey test. Fluorescence intensity data of carboxy-H_2DCFDA and protein expression of IL-1β were analyzed by the Kruskal-Wallis and Mann-Whitney tests. The variables cell viability and H_2O_2 diffusion were correlated by Pearson linear correlation analysis. All tests were set at a significance level of 5%. SPSS 19.0 software (SPSS Inc, Chicago, IL, USA) was used to run the statistical analyses.

RESULTS

Cell Viability/H_2O_2 Diffusion

Significant reductions in cell viability relative to the NC were observed for all bleached groups. The percentages of cell viability reduction were 38.7%, 38.5%, 61.7%, 39.4%, and 31.5% for the PC, RMGIC, RMGIC/HD, RC, and RC/HD groups, respectively. A significant difference from the PC was observed only for the RMGIC/HD group. No H_2O_2 was detected in the NC, which was discarded from statistical analysis. The highest H_2O_2 value was found for the RMGIC/HD group, which was significantly higher than that of the other experimental groups. Considering the PC as presenting 100% of H_2O_2 diffusion, about 125% of H_2O_2 diffusion was observed in the RMGIC/HD group. These data are demonstrated on Figure 2. The linear Pearson correlation analysis demonstrated a significant negative correlation between the variables cell viability and H_2O_2 diffusion (Pearson coefficient of correlation=−0.87, p=0.0001) (Figure 2). Therefore, the higher the H_2O_2 diffusion, the lower the cell viability.

Oxidative Stress Generation/IL-1β Protein Expression

All bleached groups presented significantly more intense fluorescence values for the carboxy-H_2DCFDA probe than did the NC group, demonstrating that in all bleached groups, the cells were under oxidative stress. For IL-1β protein expression, only RMGIC/HD presented values significantly higher than those of the NC and PC (Figure 3).

ALP Activity/Mineralized Nodule Deposition

Significant reductions in ALP activity and mineralized nodule deposition were observed for all bleached groups relative to those in the NC. A reduction of around 29% to 67% of ALP activity and 66% to 82% of mineralized nodule deposition was observed in

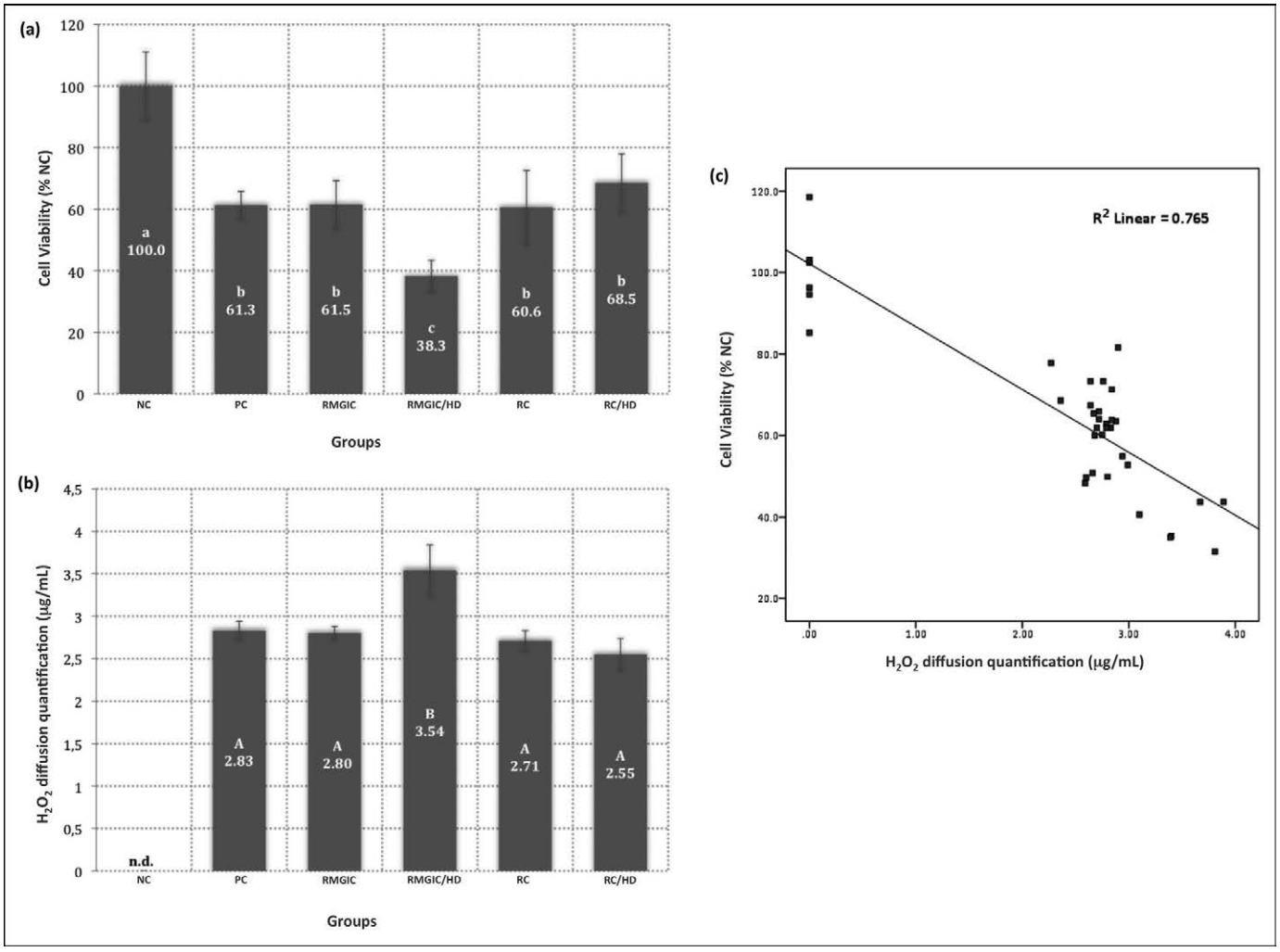

Figure 2. *Bar graph of cell viability (a) and H_2O_2 diffusion (b). Different letters indicate statistically significant differences among groups (Tukey, $p<0.05$). Values are average ± standard deviation ($n=6$). (c) The linear Pearson correlation analysis demonstrating a significant negative correlation between the variables of cell viability and H_2O_2 diffusion (Pearson coefficient of correlation=−0.87, $p=0.0001$).*

bleached groups relative to those in the NC, with RMGIC/HD being the group that featured the highest reduction values (Figure 4).

DISCUSSION

In the present investigation, a traditional in-office bleaching technique was performed on enamel/dentin discs restored with RMGIC or etch-and-rinse adhesive/RC, which were subjected or not subjected to hydrolytic degradation. The amount of H_2O_2 that reached the pulpal space was quantified, and its toxicity to cultured odontoblast-like cells was assessed. The RMGIC used in the present investigation (Vitremer) was previously assessed regarding the tooth/restoration interface quality, such as bond strength to enamel/dentin and nano/microinfiltration. The results of *in vitro* and *in vivo* studies demonstrated that Vitremer featured results similar to or better than those of other brands.[23-28] It was also demonstrated that powder/liquid RMGICs have higher microtensile bond strength to dentin and lower microleakage than do the paste/paste restorations when evaluated immediately after cavity restoration placement and following simulated hydrolytic degradation.[29,30] Therefore, the interface created with the powder/liquid Vitremer in the present study represents a challenging situation for RMGIC restorations exposed to bleaching. Regarding the adhesive system and composite resin used in the RC group, the materials and restoration techniques performed were based upon those described in a previous study[17] in which the tooth/restoration interface had no effect on transenamel and transdentinal H_2O_2 diffusion and cytotoxicity to the MDPC-23 cells, even when subjected to hydrolytic

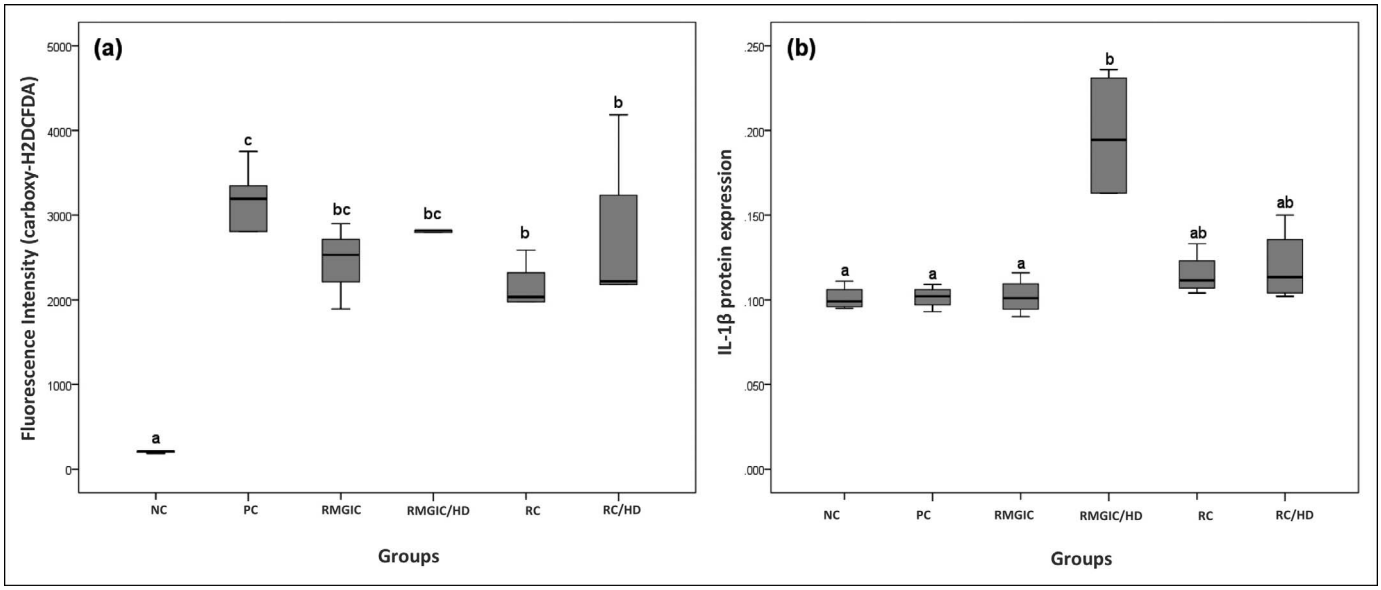

Figure 3. *Box-whisker plot of fluorescence intensity of H_2DCFDA (a), indicating the occurrence or lack of occurrence of oxidative stress and IL-1β expression (b). Different letters indicate statistically significant differences among groups (Mann-Whitney,* $p<0.05$; $n=6$).

degradation and exposed to calcium-containing 20% and 35%-H_2O_2 bleaching gels. Therefore, this group served as a control for comparison of the effect of the RMGIC interface subjected to bleaching. However, materials from only one single brand were tested in the present investigation, which represents a limitation of this study; therefore, the results should be interpreted with caution.

It has already been demonstrated[16] that the in-office bleaching protocol involving three 15-minute applications of a 35% H_2O_2 gel causes intense tooth sensitivity in sound human teeth, which is even higher in adhesive-restored teeth. Therefore, this professional bleaching technique was assessed in the present investigation to determine the role of different tooth/restoration interfaces in transenamel and transdentinal cytotoxicity to pulp cells. Regarding the sound/bleached samples (SD group), the results of the present study are in agreement with those of previous investigations,[10-12,17] in which highly concentrated H_2O_2 bleaching gels applied to sound tooth surfaces for 45 minutes caused moderate cytotoxicity, characterized by decreased cell viability from 21% to 50%.[31,32] These data have been correlated with the intense tooth sensitivity claimed by the patients subjected to traditional in-office bleaching, as observed in clinical trials.[1-5] Using a

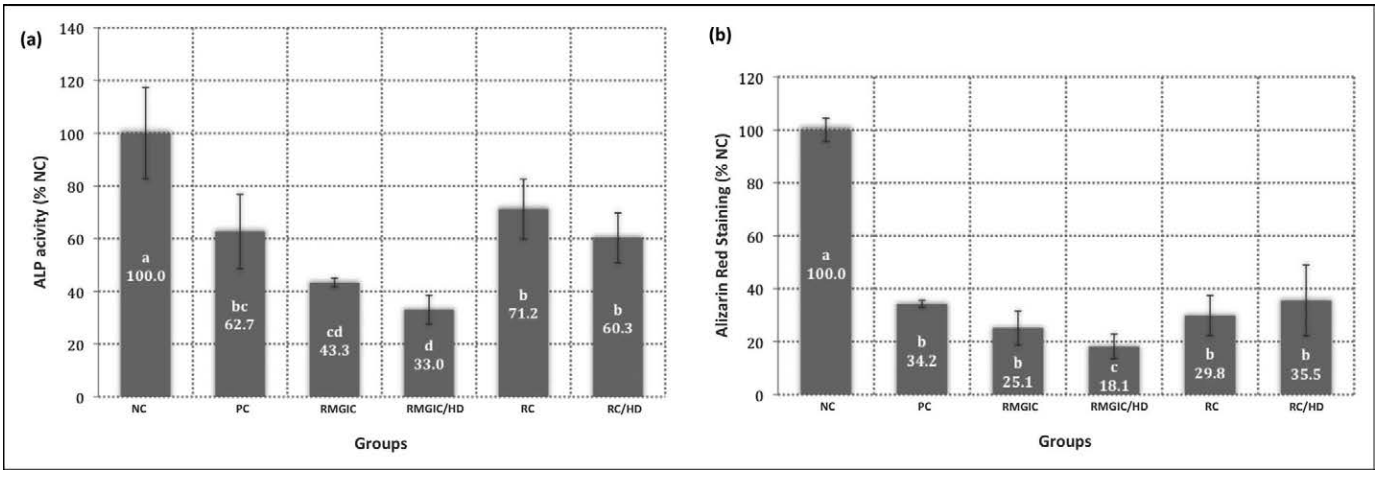

Figure 4. *Bar graph of ALP activity (a) and Alizarin Red staining (b). Different letters indicate statistically significant differences among groups (Tukey,* $p<0.05$*). Values are average ± standard deviation (n=6).*

similar experimental protocol, Soares and others[12] showed that the reduction in MDPC-23 cell viability mediated by traditional in-office protocols is related to intense oxidative stress generation, culminating with cell membrane damage and cell death by necrosis. In clinical situations, oxidative cell damage triggers an inflammatory reaction in pulp tissue,[8,9] during which cell-derived factors are released. This tissue response, associated with increased pulp pressure, may trigger impulses to the intradentinal pulpal nerve fiber endings, causing clinical symptoms of tooth sensitivity.[33] According to the literature, [8-12,17,18] the intensity of these adverse events is directly related to the amount of H_2O_2 that reaches the pulp chamber.

Analysis of the data obtained in the present study also demonstrated that cells exposed to the extracts obtained from bleached enamel/dentin discs were under intense oxidative stress; the fluorescence intensity of carboxy-H_2DCFDA probe increased about 10 to 15 times in bleached groups compared with that in the NC, associated with significant cell viability reduction (from 31.5% to 61.7%). Oxidative stress arises when the balance between the production of ROS and their neutralization by antioxidant systems is disturbed.[34,35] Depending on the intensity of the oxidative stress, the cell components undergo severe oxidative damage, ultimately compromising cell viability.[12] It has been reported[34-36] that ROS accumulated during oxidative stress are transient as a result of their high reactivity, leading to oxidative damage of indispensable biomolecules such as proteins, lipids, and nucleic acids. Therefore, the H_2O_2-mediating reduction in pulp cell viability has been related to two basic mechanisms: 1) induction of oxidative stress conditions due to H_2O_2 diffusion through cell membranes and 2) direct contact of H_2O_2 by-products with cell membranes, causing disruption and cell death.[12,34-36] Associated with these results, significantly decreased ALP activity (from 29% to 57%) and mineralized nodule deposition (from 65% to 82%) were observed at seven days post–bleaching treatment compared with the NC.

The regulation of the odontoblastic phenotype is critical for the homeostasis of the pulp-dentin complex. Under a noxious stimulus of mild intensity, upregulation of odontoblastic secretory activity is observed, which is accomplished by deposition and mineralization of collagen-rich matrix in a process called reactionary dentinogenesis. During this process, several proteins are expressed by odontoblasts, such as DSPP, DMP-1, and ALP.[37] The ALP is considered an initial marker for *in vitro* analysis of the odontoblastic maturation process, since the activity of this protein provides the phosphate needed for the biomineralization process. Nevertheless, mineralization nodule deposition (MND) is considered a late marker, since it demonstrates the capability of mature odontoblasts to deposit and mineralize the organic matrix.[38,39] Recently, Lee and others[40] demonstrated that the toxic concentrations of H_2O_2 in contact with human pulp cells caused intense oxidative stress and cell viability reduction associated with the downregulation of odontoblastic marker expression and the inhibition of odontoblastic differentiation. Also, as determined in the present study, toxic concentrations of H_2O_2 in contact with odontoblast-like cells for a relatively short-term period (one hour) caused intense reductions in ALP activity and MND. Intense reductions in ALP activity by MDPC-23 cells after one-hour exposure to components released from 35%-H_2O_2 gel applied for 45 minutes to enamel/dentin discs were also shown previously.[10] Therefore, traditional in-office bleaching may drastically alter the regenerative capability and homeostasis of pulp tissue.

In the present study, the most intense cell alteration was observed for the RMGIC/HD group, which featured cell viability reduction that was 1.6 times higher, and ALP activity and MND that were around 1.8-1.9 times lower, than in the sound/bleached (SD) group. In addition, in this RMGIC/HD group, higher IL-1β expression was observed compared with that in the other experimental and control groups. One may suggest that the intense cell alterations observed in this study for the RMGIC/HD group were caused by the higher diffusion of H_2O_2 (about 25%) than was observed in the sound/bleached group. Pro-inflammatory cytokines, such as IL-1β, are barely expressed or not expressed in healthy human dental pulp, but their expression level is enhanced in inflamed pulps or after *in vitro* exposure of odontoblasts to lipopolysaccharides.[41-43] The higher the expression of pro-inflammatory cytokines, the higher the intensity of tissue damage in the *in vivo* situation, since pulp cells under inflammatory reaction increase the expression of proteolytic enzymes, promoting disruption of the extracellular matrix.[44,45] This histological event, characterized by inflammatory reactions associated with partial tissue necrosis and the absence of tertiary dentin deposition, was reported by de Souza Costa and others[8] after applying a bleaching gel with a high concentration of H_2O_2 to human sound teeth for 30 minutes. This effect has also been observed in previous *in vitro* studies, in which pulp cells exposed

to pro-inflammatory cytokines at high concentrations for long periods impaired odontoblastic marker expression and the deposition of mineralized matrix.[46-48] Treatment of mesenchymal pulp stem cells from pulp tissue with IL1-β for 48 hours significantly reduced the expression of odontoblastic markers and MND, demonstrating that this cytokine significantly affected odontoblastic differentiation capability.[49]

In groups restored with etch-and-rinse adhesive/RC, no significant difference, compared with the sound/bleached group, was observed for all cell analyses performed, nor was any difference observed for H_2O_2 diffusion. Significant correlation between the amount of H_2O_2 diffusion and cytotoxicity was demonstrated by Pearson correlation analysis; therefore, the higher the H_2O_2 diffusion, the lower the cell viability. One can conclude that the exposure of RMGIC restorations to hydrolytic degradation created an interface pathway for H_2O_2 diffusion. Therefore, the null hypothesis was partially rejected, since the RMGIC restoration, subjected to hydrolytic degradation, had a significant effect on the indirect cytotoxicity of the 35%-H_2O_2 bleaching gel to pulp cells. It has already been demonstrated[20,21] that the shear bond strength of RMGIC to tooth structure is significantly lower than that observed for etch-and-rinse/RC restorations. Consequently, the nano/microleakage of RMGIC is highly affected by hydrolytic degradation.[50,51] A clinical study with cervical restorations of different brands of RMGICs demonstrated that Vitremer scored the highest percentage of intact margins after 18 months relative to other RMGICs; however, this material showed significant reductions in perfect margin adaptation (48%) and microleakage (26%) after six months in the oral cavity. An 18-month period of restoration margin evaluation showed that material fragments crumbled off at the interface, leaving clear local defects.[23] Additionally, Yu and others[22] demonstrated that the tooth-bleaching procedure increased the microleakage at the RMGIC interface subjected to hydrolytic degradation by a factor of 3, whereas no alteration was observed for etch-and-rinse adhesive/RC restorations. According to the authors, the marginal gaps at the RMGIC interface favor H_2O_2 diffusion to cause oxidation on both the restoration[52-54] and the tooth structure,[55,56] as previously demonstrated. In the present study, the RMGIC interface not subjected to hydrolytic degradation did not allow for more intense H_2O_2 diffusion or cytotoxicity to the pulp cells. Khoroushi and Fardashtaki[57] also observed that the microleakage of class V RMGIC restorations (Vitremer), subjected only to 500 cycles of thermocycle regime, was not affected by a 38%-H_2O_2. According to the authors, an appropriate bond in the cavity margins protects restoration from the risk of peroxide penetration during the bleaching procedure, as those promoted by new and nondegraded RMGIC restorations.[57] It is important to mention that the data obtained in this study should be interpreted with caution, since only the conventional RMGIC interface was tested. Previous studies showed that the inclusion of hydroxyapatite (HA) or bioactive glasses to RMGICs may result in the deposition of HA at the restoration interface.[58] Although several investigators have reported that the inclusion of these bioactive substances has little or no effect on bond strength to dentin and microinfiltration,[59-61] one study has reported that these materials have a protective effect when dentin is subjected to demineralization, leading to the deposition of HA at the tooth/restoration interface.[61,62] Therefore, future studies should be performed to assess the effects of bleaching agents on the bioactive RMGICs tooth/restoration interface and pulp cell cytotoxicity.

The more resistant interface created with etch-and-rinse/RC restorations may act as a barrier for H_2O_2 diffusion, and no significant alteration in the tooth/restoration interface takes place after bleaching.[22] Previous studies[63-66] have demonstrated that the quality of the adhesive interface has a significant effect on H_2O_2 susceptibility and that different adhesive systems have variable degrees of susceptibility, as follows: one-step self-etch > two-step self-etch > etch-and-rinse systems. Soares and others[17] did not report differences in H_2O_2 diffusion and its cytotoxicity to MDPC-23 cells when the bleaching gel was applied to sound or etch-and-rinse/RC restored enamel/dentin discs, as observed in the present investigation. However, the authors[18] described the occurrence of higher cytotoxicity when the same bleaching gel was applied to two-step self-etch adhesive/RC restored discs compared with sound/bleached discs.

Bonafé and others[16] observed that teeth containing old adhesive restorations with no clinically perceptible margin degradation and subjected to bleaching presented more intense tooth sensitivity compared with that of sound and bleached teeth. However, the authors had no control over the materials and application protocols used to perform the adhesive restorations. This same situation may be frequently experienced in the clinical dental practice. Some authors[22] have suggested the application of a resin coating around the restoration

margins to avoid the contact of bleaching gels with the tooth/restoration interface. According to Yu and others,[22] the protection of RMGIC restoration margins with resin prevented bleaching-induced microleakage *in vitro*. The authors observed no visually detectable color difference between the coated and uncoated tooth surfaces after bleaching. Therefore, this method seems to be an interesting alternative to be assessed to verify its protective effect on bleaching-induced tooth sensitivity/pulp cell damage in restored teeth.

Finally, according to the results of the present investigation, it is possible to affirm that a degraded tooth/RMGIC interface may act as a pathway for H_2O_2 diffusion through the tooth structure, indirectly influencing pulp cell cytotoxicity. It is known that the association of H_2O_2 and resin monomers results in increased cytotoxicity to pulp cells *in vitro*;[67,68] however, the effects of H_2O_2 and RMGIC components on the increased toxicity observed in the present investigation are unknown. Also, it is important to note that only the in-office bleaching protocol with the three 15-minute applications of 35%-H_2O_2 gel to sound and restored teeth was evaluated in the present investigation. It is well known that H_2O_2 diffusion,[6,7,10-12] pulp cell toxicity,[10-12] and the tooth sensitivity intensity/prevalence[1-5] are directly related to the bleaching protocol. Therefore, analysis of the data obtained in the present study can guide clinicians to an understanding of the risks involved in the application of bleaching gels to restored teeth. However, the toxic effects of at-home and other alternative professional bleaching protocols on pulp cells should be addressed in future studies.

CONCLUSION

According to the results found in this investigation, the presence of a tooth/RMGIC hydrolytically degraded interface favors transtooth H_2O_2 diffusion after three 15-minute applications of a 35%-H_2O_2 gel to its surface, which may increase its toxic effects on pulp cells.

Acknowledgments

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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 Departamento de Fisiologia e Patologia Faculdade de Odontologia de Araraquara - UNESP.

Conflict of Interest

The authors 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 Storage Time on Bond Strength and Nanoleakage Expression of Universal Adhesives Bonded to Dentin and Etched Enamel

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Clinical Relevance

The bond strength of a universal adhesive to dentin or etched enamel can be similar to that of conventional restorative systems in the long term; however, the bonding efficacy of both bonding techniques may decrease with aging, leading to leakage formation at the adhesive/dentin interface.

SUMMARY

Purpose: To investigate bond strength and nanoleakage expression of universal adhesives (UA) bonded to dentin and etched enamel.

Methods: Extracted human third molars were sectioned and ground to obtain flat surfaces of dentin (n = 36) and enamel (n = 48). Dentin and etched enamel surfaces were bonded with one of two UAs, All-Bond Universal (ABU) or Scotchbond Universal (SBU); or a two-step self-etching adhesive, Clearfil SE Bond (CSEB). A hydrophobic bonding resin, Adper Scotchbond Multi-Purpose Bond (ASMP Bond) was applied only on etched enamel. Following each bonding procedure, resin composite blocks were built up incrementally. The specimens were sectioned and subjected to microtensile bond strength (MTBS) testing after 24 hours or one year water storage, or immersed into ammoniacal silver nitrate solution after aging with 10,000 thermocycles and observed using scanning electron microscopy. The per-

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centage distribution of silver particles at the adhesive/tooth interface was calculated using digital image-analysis software.

Results: The MTBS (CSEB = SBU > ABU, for dentin; and CSEB > ABU = SBU = ASMP Bond, for etched enamel) differed significantly between the adhesives after 24 hours. After one year, MTBS values were reduced significantly within the same adhesive for both substrates (analysis of variance, Bonferroni *post hoc*, $p<0.05$), and no significant differences were found among the adhesives for etched enamel. Silver particles could be detected within the adhesive/dentin interface of all specimens tested. Kruskal-Wallis mean ranks for nanoleakage in ABU, SBU, and CSEB were 16.9, 18.5 and 11, respectively ($p>0.05$).

Conclusions: In the short term, MTBS values were material and dental-substrate dependent. After aging, a decrease in bonding effectiveness was observed in all materials, with nanoleakage at the adhesive/dentin interface. The bonding of the UAs was equal or inferior to that of the conventional restorative systems when applied to either substrate and after either storage period.

INTRODUCTION

The ultimate goal of adhesive dentistry is to provide simple and fast adhesive application with durable bonding to enamel and dentin.[1] Self-etching adhesives contain acid monomers to enable etching of dental structures, hydrophilic monomers to enhance wettability, and hydrophobic bond resin monomers that infiltrate into the demineralized rough enamel or porous dentin surface,[2] providing monomer conversion and strengthening of the tooth-resin interface. Self-etching primer systems combine the etching and the priming steps into one, whereas all-in-one systems combine a self-etching primer and a bonding agent into one application step.

Among the self-etching adhesives available in the market, there is a growing interest in multi-mode adhesives, or so-called universal adhesives (UA). They are designed with the same concept as all-in-one adhesives but incorporate the versatility of being adaptable to different clinical situations.[3] According to the manufacturers, this new category of simplified one-bottle adhesives is indicated for direct and indirect restorations without the need for a primer. This adhesive can be applied to dentin or enamel using a self-etching approach or after selectively etching the enamel. Some UAs contain 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer in their formulation. This functional monomer is considered one of the most effective monomers with regard to chemical interaction and durability.[4,5]

Although the initial bonding performance of UAs to dentin seems to be material and technique dependent,[6,7] recent studies have reported good long-term performance of this new category of adhesives.[3,8] In addition, it has been suggested that similar to other self-etching adhesives,[9] pre-etching the enamel increases the bond strength values for UAs, based on a short-term evaluation.[10-12] Despite promising results, there are only a few studies with regard to the bonding effectiveness of MDP-containing UAs compared with those investigating conventional restorative systems bonded to dentin and etched enamel structures.[13]

Nanoleakage is leakage at nanometer-sized channels, which can occur within the hybrid layer and/or in the adhesive layer, in the absence of marginal gaps.[14,15] This phenomenon has been widely implicated as an important factor that leads to the degradation of the bonding to dental tissue.[14-16] It may be caused by insufficient infiltration of resin into the demineralized collagen network or by incomplete polymerization of hydrophilic monomers in the submicron interfacial spaces. The unprotected collagen fibrils that result from this may be vulnerable to degradation by oral and bacterial enzymes.[17,18] Under *in vitro* evaluations, interfacial sealing, bond strength, and artificial aging appear to be related to one another,[19] and these can be considered potential clinical predictors of the success of a restoration.[20]

Therefore, the aim of this study was to compare the MTBS and nanoleakage expression of two UAs currently in use with those of a conventional two-step self-etching adhesive to dentin and etched enamel substrates. In addition, on etched enamel substrate, use of UAs was also compared with a conventional technique consisting of a hydrophobic bonding resin applied directly to etched enamel. The null hypotheses tested were 1) there is no significant difference in bond strength between the materials tested after 24 hours or one year of water storage when bonded to dentin or etched enamel; and 2) after 10,000 cycles of thermal stress, the use of UAs does not result in more nanoleakage along the adhesive/tooth substrate interface compared with the conventional adhesives tested.

Table 1: *Materials Used in This Study*

Adhesive System (Batch Number)	Composition	Dentin Self-etch Mode	Enamel Etch-and-rinse Mode
All-Bond Universal (1200003968); BISCO, Schaumburg, IL, USA	Adhesive: MDP, Bis-GMA, HEMA, ethanol, water, initiators	Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10-15 sec per coat. Evaporate excess solvent by thoroughly air-drying with an air syringe for 10 sec until there is no visible movement of the material. Light cure for 10 sec.	Apply etchant for 15 sec. Rinse for 10 sec. Remove excess water with absorbent pellet for 2 sec. Apply adhesive as for the self-etching mode.
Scotchbond Universal (472387); 3M ESPE, St Paul, MN, USA	Adhesive: MDP, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	Apply adhesive to the preparation with a microbrush and rub it in for 20 sec. Gently air blow-dry for 5 sec until there is no visible movement of the material. Light cure for 10 sec.	Apply etchant for 15 sec. Rinse for 10 sec. Air dry for 2 sec. Apply adhesive as for the self-etching mode.
Clearfil SE Bond (Primer: 01115A Bond: 01671A); Kuraray Noritake Dental Inc, Tokyo, Japan	Primer: MDP, HEMA, camphorquinone, hydrophilic dimethacrylate, water Bond: MDP, Bis-GMA, HEMA, camphorquinone, hydrophobic dimethacrylate, *N,N*-diethanol *p*-toluidine bond, colloidal silica	Apply primer to tooth surface and leave in place for 20 sec. Blow-dry. Apply bond to the tooth surface and then create a uniform film using a gentle air flow. Light cure for 10 sec	Apply etchant for 15 sec. Rinse for 10 sec. Air dry. Apply adhesive as for the self-etching mode.
Adper Scotchbond Multi-Purpose Bond (N205453); 3M ESPE, St Paul, MN, USA	Bond: Bis-GMA, HEMA, triphenylantimony	N/A	Apply etchant for 15 sec. Rinse for 10 sec. Air dry. Apply adhesive with slight agitation for 10 sec. Air dry. Apply adhesive. Light cure for 10 sec.

Abbreviations: Bis-GMA, bisphenol-A diglycidyl ether dimethacrylate; HEMA, hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; N/A: Not applicable (because Adper Scotchbond Multi-Purpose Bond was applied only on etched enamel substrate).

METHODS AND MATERIALS

Specimen Preparation

A total of 84 extracted intact human third molars were used according to the guidelines of the local Ethics Committee, under protocol number 143/2014. For dentin specimens (n=36), the occlusal one-third and the root of each tooth were cut using a diamond saw (IsoMet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. The exposed coronal flat dentin surface was polished with 600-grit silicon carbide paper (Norton, Vinhedo, Brazil) under running water to ensure that enamel isles were completely removed.

For enamel specimens (n=48), the root of each tooth was removed and a mesiodistal cut perpendicular to the middle occlusal surface was performed using a diamond saw (IsoMet, Buehler Ltd) under water cooling. Flat buccal and lingual enamel surfaces were obtained from 48 teeth after wet-grinding with 600-grit silicon carbide paper (Norton). All the specimens for enamel substrate were pre-etched with 35% phosphoric acid for 15 seconds (Scotchbond Universal Etchant; 3M ESPE, St Paul, MN, USA), rinsed thoroughly, and air-dried before each adhesive application.

The obtained dentin or etched enamel flat surfaces were assigned randomly to three groups (n=12 per adhesive/substrate) according to the material used: two UAs, All-Bond Universal (ABU; Bisco, Schaumburg, IL, USA) and Scotchbond Universal (SBU; 3M ESPE); or a two-step self-etch adhesive, Clearfil SE Bond (CSEB; Kuraray Noritake Dental Inc, Tokyo, Japan). Only on etched enamel substrate, for an additional group (n=12), consisting of a hydrophobic bonding resin, Adper Scotchbond Multi-Purpose Bond (ASMP Bond; 3M ESPE) was applied directly to the substrate. After each bonding procedure, all the specimens were restored with a composite resin, Filtek Z350XT (3M ESPE) in two increments of 2 mm each. Each increment was cured for 40 seconds using a quartz-tungsten-halogen light-curing unit (Optilux 501, Kerr, CA, USA; 600 mW/cm^2 intensity). The specimens were prepared according to each of the manufacturer's instructions (Table 1).

MTBS Measurement and Fracture Analysis

After being stored for 24 hours in water at 37°C, 10 restored teeth from each adhesive and each substrate (dentin: n=30; etched enamel: n=40) were sectioned serially using a low-speed diamond saw

(IsoMet, Buehler Ltd) under water cooling to produce parallel piped sticks (0.9 mm wide × 0.9 mm thick × 6 mm length) with their long axis perpendicular to the bonded interface. Half the number of sticks from each tooth and each substrate were selected randomly for immediate testing, and the remainder were stored for one year in distilled water.

For the MTBS testing, the ends of the sticks were carefully fixed with cyanoacrylate glue (Model Repair II Blue, Sankin Industry Co, Tokyo, Japan) to a jig in a universal testing machine (EZ Test, Shimadzu, Kyoto, Japan) and subjected to a tensile force at a crosshead speed of 1 mm/min. After the bond strength testing, the two ends of the fractured surfaces were mounted on brass stubs, gold-coated, and observed using scanning electron microscopy (SEM; JSM5600, JEOL Ltd, Tokyo, Japan) at magnifications of 100× and 1000×.

For dentin specimens, the failure mode of each beam was determined by two experienced researchers and then classified, by consensus, into categories: cohesive failure in composite resin (C); failure between composite resin and adhesive (CA); failure between adhesive and dentin (AD); mixed failure of composite resin, adhesive, and dentin (CAD); cohesive failure in adhesive (A); cohesive failure in hybrid layer (HL); and cohesive failure in dentin (D).

In a similar way, for etched enamel specimens, the failure mode of each beam was determined and classified into cohesive failure in composite resin (C); failure between composite resin and adhesive (CA); failure between adhesive and etched enamel (AE); mixed failure of composite resin, adhesive, and etched enamel (CAE); cohesive failure in adhesive (A); failure between enamel and dentin (ED); and cohesive failure in enamel (E).

Thermocycling Procedure

In addition, two teeth from each adhesive and each substrate were selected to undergo thermocycling. The specimens were fatigued with 10,000 thermocycles between 5°C and 55°C at a dwell time of 30 seconds per temperature and a transfer time of 10 seconds between baths (MSCT 3, Marnucci ME, Sao Carlos, Brazil).

Nanoleakage Evaluation

After the thermocycling procedure, the two teeth from each material and each substrate were vertically sectioned with a diamond saw (IsoMet, Buehler Ltd) under water coolant, across the adhesive/tooth substrate interface, into approximately 1-mm-thick slabs. Two central slabs were chosen from each tooth, forming a total of four specimens per material and per substrate. All the specimens were coated with two layers of nail varnish applied 1 mm from the bonded interface, followed by immersion into 50% (wt/vol) ammoniacal silver nitrate solution for 24 hours. Thereafter, they were rinsed thoroughly under running tap water and exposed to photo-developing solution for eight hours under fluorescent light to reduce the penetration of the ammoniacal silver nitrate into metallic silver grains. Each slab was then wet-polished with 1200-grit silicon carbide paper (Norton) and sonicated for five minutes to remove the superficial silver adsorption.

Following air-drying, the specimens were gold-coated and examined by SEM in back-scattered electron mode and energy dispersive x-ray spectroscopy (EDS; JSM5600, JEOL Ltd) at a magnification of 2000×. Interfacial images were obtained from each specimen (n=10). The percentage distribution of metallic silver particles at the adhesive/tooth substrate interface was calculated with a digital image-analysis software (NIH ImageJ 1.60, Scion, Frederick, MD, USA) in a selected area on each image at 500× (20.2 × 250 μm, height × width). Initial energy spectra analyses were performed to determine the elemental composition of the entire area. In addition, select surface areas were mapped for elements including silver, calcium, and silicon.

Statistical Analysis

The MTBS data were statistically analyzed using a three-way analysis of variance (ANOVA) with the significance level defined as $\alpha = 0.05$; bond strengths to dentin or etched enamel were dependent variables, and the adhesive, the storage period, and the substrate were factors. A Bonferroni *post hoc* test with UNIANOVA syntax was used for multiple comparisons with significant differences in bond strength means. The nanoleakage data were statistically analyzed with a Kruskal-Wallis test, with the statistical significance defined as $\alpha = 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS for Windows, Version 16.0, SPSS Inc, Chicago, IL, USA).

RESULTS

The means and standard deviations of dentin and etched enamel MTBS values obtained in this study are presented in Table 2. The three-way ANOVA showed that the bond strength results for dentin and

Table 2: *Mean MTBS of Different Adhesives Applied in a Self-etch Mode on Dentin Substrate and in an Etch-and-rinse Mode in Enamel Substrate*

Material	Dentin Substrate		Etched Enamel Substrate	
	After 24 h Water Storage	After One y Water Storage	After 24 h Water Storage	After One y Water Storage
All-Bond Universal (ABU)	39.9 (11.9) A, a	25.1 (13.2) A, b	40.8 (5.9) A, c	30.7 (9.4) A, d
Scotchbond Universal (SBU)	63.1 (8.2) B, a*	45.9 (8.4) B, b**	40.7 (13.0) A, c*	28.4 (10.8) A, d**
Clearfil SE Bond (CSEB)	63.4 (9.2) B, a	44.1 (4.2) B, b**	59.7 (13.8) B, c	27.1 (5.7) A, d**
Adper Scotchbond Multi-Purpose Bond (ASMP Bond)	N/A	N/A	40.4 (7.7) A, a	24.4 (6.5) A, b

Abbreviation: N/A: Not applicable (because ASMP Bond was tested only on etched enamel substrate).
[a] *Data are presented as the mean (standard deviation) in megapascals (MPa; n = 10 teeth). Identical capital letters in a column indicate the absence of any statistically significant difference. Identical lowercased letters in a row within the same substrate between after 24 h and one y water storage indicate the absence of any statistically significant difference. Comparisons within the same material and storage period between different substrates, and marked with one asterisk for 24 h and two asterisks for one y water storage are statistically significant. (Analysis of variance and Bonferroni* post hoc *test; significance at* $p<0.05$).

etched enamel were influenced significantly by the adhesive used, by the storage period, and by the substrate. The interaction of these three factors was not significant ($p=0.055$). On the other hand, significant statistical interaction was observed between adhesive material and storage period ($p=0.011$) and between adhesive and substrate ($p<0.001$), but no significant statistical interaction was found between storage period and substrate ($p=0.710$).

A Bonferroni *post hoc* test revealed the presence of statistically significant differences between the MTBS results after 24 hours or one year of water storage for both substrates. For dentin, CSEB and SBU showed higher bond strength values than those of ABU after 24 hours or one year of storage. However, there was a significant decrease in MTBS values within all adhesives tested after one year of storage ($p<0.05$). For etched enamel, CSEB showed significantly higher bond strength values after 24 hours; no significant difference was observed among ABU, SBU, and ASMP Bond at this time point. After one year of water storage, a significant decrease in bond strength was observed within all adhesives compared with their baselines, and the bond strength values did not differ from each other among all adhesives ($p>0.05$). Using ABU, there were no significant MTBS differences between the dentin and etched enamel when the substrates were compared after the same storage period. For SBU, the bonding to dentin did in fact result in a significantly higher bond strength than etched enamel after 24 hours. The same was true when using both SBU and CSEB after one year of water storage.

The failure modes of the tested groups are summarized in Figure 1. Representative high magnification SEM micrographs of the fracture mode patterns are shown in Figure 2. For dentin, the C failure mode predominated for SBU and CSEB at both time points, whereas ABU showed an increased incidence of AD mode of failure after one year storage. For etched enamel, a slight increase of C and CAE mode of failure was observed in most of the materials in the long term.

Representative images of silver-challenged specimens for dentin and etched enamel substrates after 10,000 thermocycles are illustrated in Figures 3 and 4, respectively. High magnification of SEM micrographs after the silver challenge revealed the existence of nanoleakage formation only for dentin specimens after 10,000 thermocycles. Images in which the total percentage distribution of silver tracer within the interface was calculated are shown in Figure 3. For the nanoleakage test, no significant statistical difference was observed among all adhesives after 10,000 thermocycles (Kruskal-Wallis test, $p>0.05$). A distinctive silver-spotted pattern of nanoleakage formation could be recognized along the adhesive/dentin interface in all specimens, and its silver percentage distribution means and mean ranks can be visualized in Table 3. In addition, SEM/EDS images of the interface with silver particles are shown in Figure 5. Elemental silver was identified by EDS analysis, confirming the results obtained.

DISCUSSION

In this study, the bonding strength of UAs was tested on dentin and etched enamel and compared with a conventional two-step self-etching adhesive. The MTBS test was chosen owing to its advantages over other bond strength tests: Bonding measurement can be achieved for very small areas, and multiple beams can be obtained from a single tooth.[21] It also allowed us to divide the number of beams obtained from each specimen and to evaluate them at two storage times.

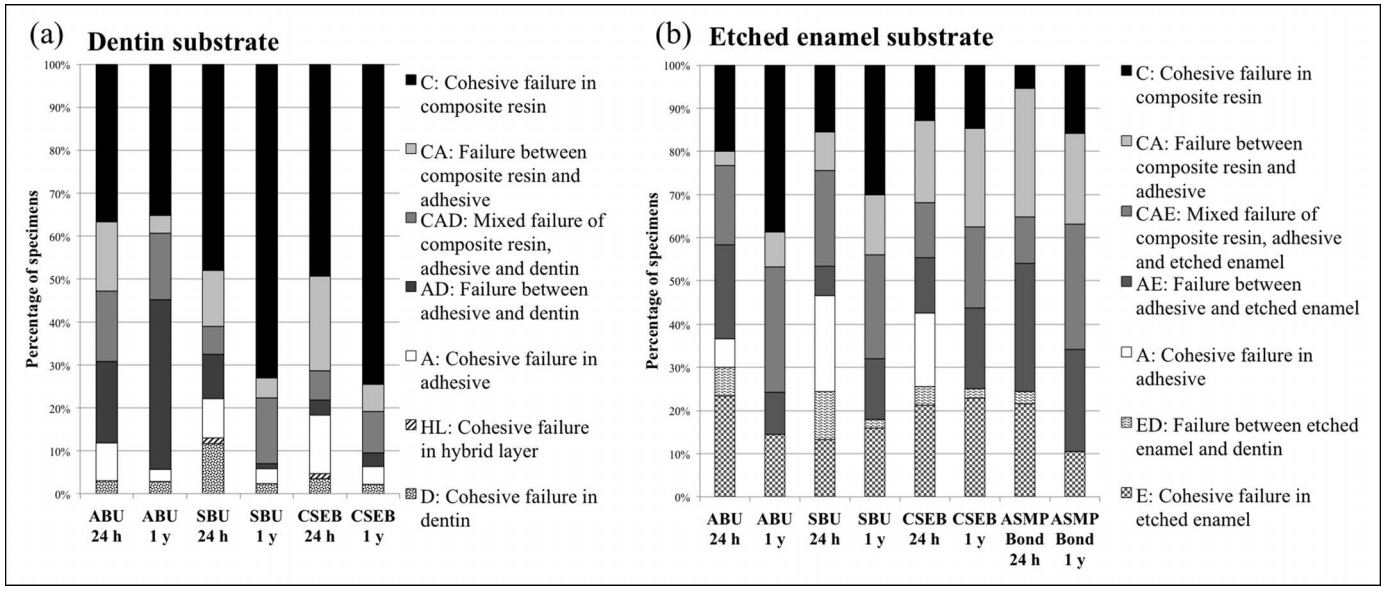

Figure 1. *Distribution (%) of failure modes of the adhesive materials tested after 24 hours (24 h) and one year (1 y) water storage in (a): dentin and (b): etched enamel substrates.*

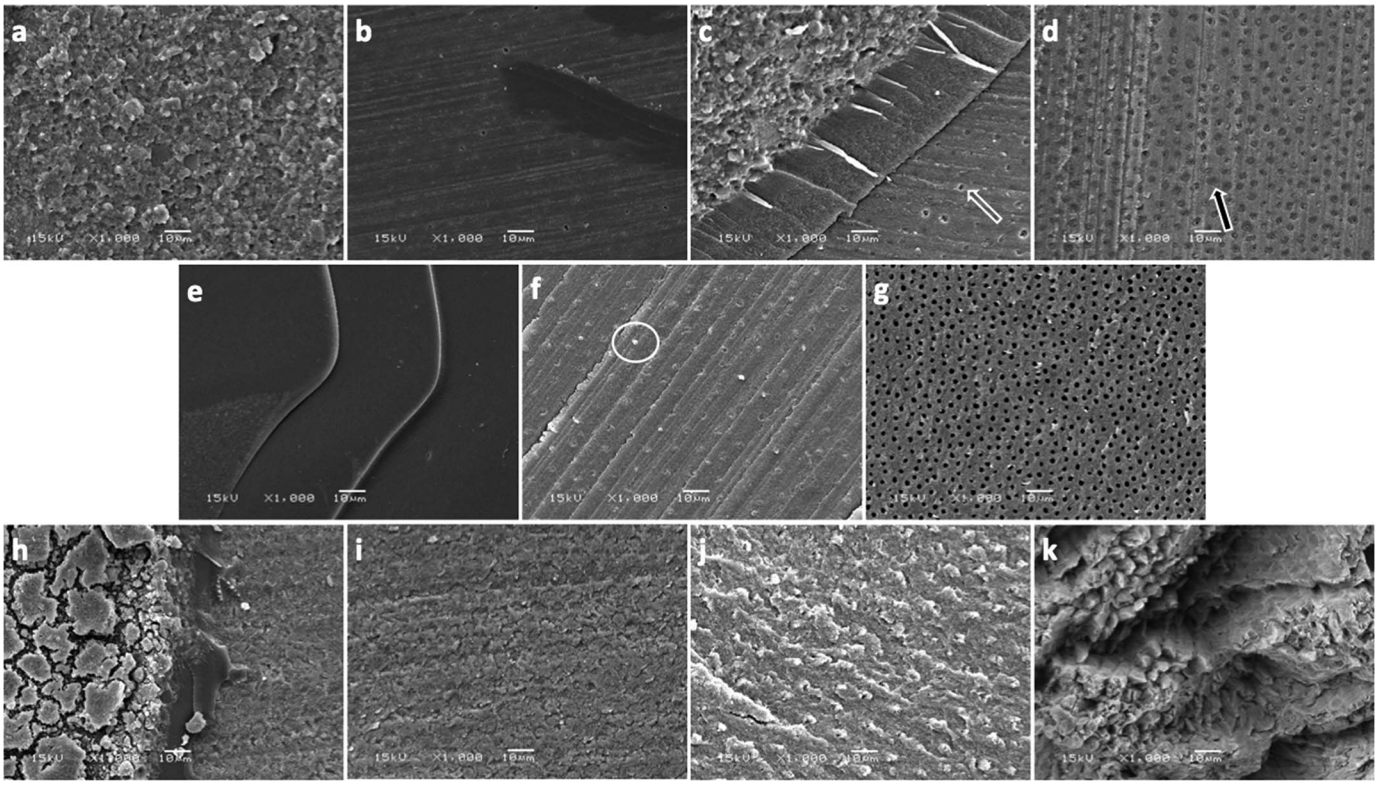

Figure 2. *SEM (1000×) showing representative fracture patterns on dentin (a-g) and etched enamel (h-k) substrates. Images are ordered as follows: (a): cohesive failure in composite resin, C; (b): failure between composite resin and adhesive, CA; (c): mixed failure of composite resin, adhesive, and dentin, CAD; (d): failure between adhesive and dentin, AD; (e): cohesive failure in adhesive, A; (f): cohesive in hybrid layer, HL; (g): cohesive failure in dentin, D; (h): mixed failure of composite resin, adhesive, and etched enamel, CAE; (i): failure between adhesive and etched enamel, AE; (j): failure between etched enamel and dentin, ED; and (k): cohesive failure in etched enamel, E. Demineralized dentinal tubules can be observed in (c) (arrow). Smear plugs in dentinal tubules can be visualized in (d) (black arrow). Circled area in (f) shows complete resin infiltration in the dentinal tubules.*

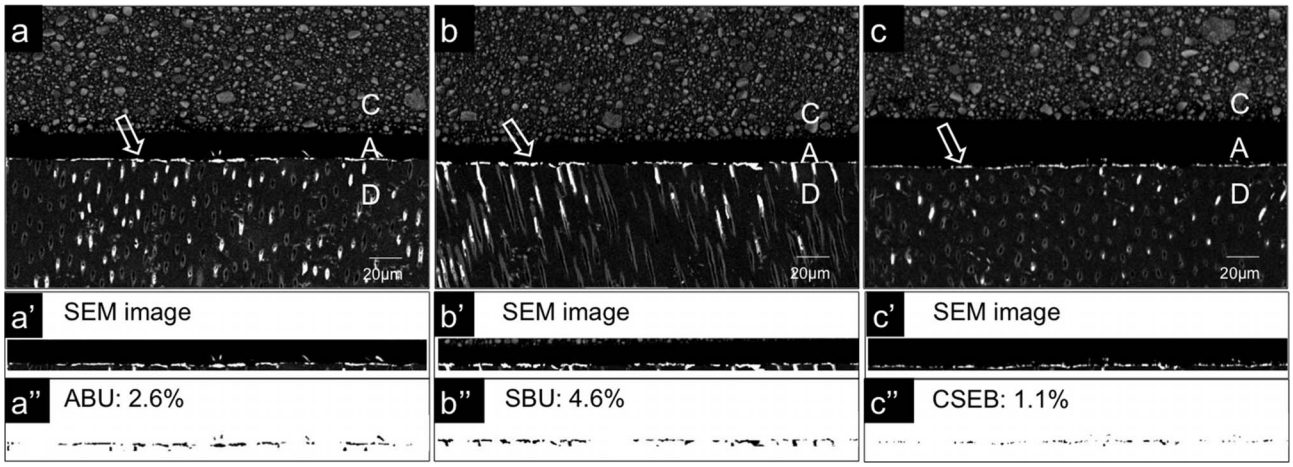

Figure 3. *Representative back-scattered scanning electron micrographs of nanoleakage at the adhesive/dentin interface in (a–c) after 10,000 thermocycles, the corresponding selected interfacial area (a′–c′), and the scored binary image obtained by the digital image analysis software (a″–c″). Images are ordered as follows: (a) ABU; (b) SBU; (c) CSEB. (a–c) Spotted silver patterns can be visualized in all the adhesives (arrow). (a′–c′) Standardized selected interfacial areas to be analyzed by the digital image analysis software. (a″–c″) The selected image was converted to a binary image to distinguish the silver area (black target pixels) from the resin and dentin (white background pixels). Percentage (%) distribution of metallic silver particles at the selected interface of the scanning electron micrograph image was calculated by the digital image analysis software; the results are indicated on each binary image: (a″) ABU; (b″) SBU; (c″) CSEB. Abbreviations: C, composite resin; A, adhesive; D, dentin.*

In addition, specimens were subjected to thermal aging prior to the nanoleakage test, and interfacial analysis for silver deposits was performed using SEM/EDS images. The use of 10,000 thermal cycles has been suggested to correspond to approximately one year of *in vivo* functioning.[22] EDS can produce quantitative and qualitative analysis of various elements' distribution and is considered to be a sensitive and accurate chemical component detection method.[23] Using EDS, the presence of elemental silver at specific locations could be confirmed. In this way, it was less likely that the investigators would misinterpret brightness caused merely by the electron microscope edge effect as indicative of silver particles.[24] Besides, this study used a digital image-analysis software to score the percentage of silver tracer particles within the adhesive/dentin interface. The percentage of silver particles within a selected area was calculated on the basis of the contrast and brightness of each pixel on the digital image.[25]

In the current study, ABU, SBU, and CSEB were bonded to dentin and etched enamel substrates, and their compositions include MDP as functional monomer. MDP has been reported to interact chemically with hydroxyapatite and to form a hydrolytically stable bond with calcium.[4,26] Indeed, no significant statistical difference of MTBS values was observed between SBU and CSEB for dentin substrate after 24 hours or one year of water storage; however, significantly lower bond strength was found for ABU. Conflicting results have been reported regarding the bond performance of ABU when applied in a self-etch mode.[3,6,7] Potentially, the ultramild acidity (pH=3.1)[13] of this material compared with that of SBU (pH=2.7)[13] and CSEB primer (pH=2.1)[6] may have limited the penetration of ABU's resin monomers into the dentinal tubules and intertubular dentin. In addition, ABU, in its formulation, contains bisphenol-A diglycidyl methacrylate (Bis-GMA), which is a highly viscous monomer.[27] It has been

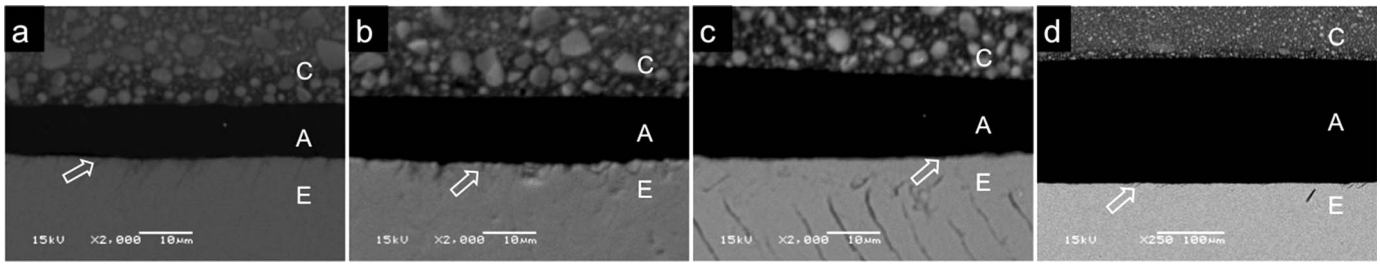

Figure 4. *Representative back-scattered SEM micrographs of nanoleakage at the adhesive/etched enamel interface in (a): ABU; (b): SBU; (c): CSEB; and (d): ASMP Bond after 10,000 thermocycles. Silver particles could not be visualized at the interface. Resin tags are indicated with the arrow. Abbreviations: C, composite resin; A, adhesive; D, dentin.*

Table 3: *Mean and Mean Ranks of Silver Penetration Values (%) at the Adhesive/Dentin Interface of Different Adhesive Systems*

Material	Dentin Substrate	
	After 10,000 Thermocycles	
	Mean	Mean Ranks
All-Bond Universal (ABU)	2.2 (1.1)	16.9 A
Scotchbond Universal (SBU)	4.4 (3.8)	18.5 A
Clearfil SE Bond (CSEB)	1.1 (1.0)	11.0 A

[a] *Data are presented as the mean (standard deviation) and the mean ranks. Identical small-cap letters in the column of mean ranks values indicate the absence of any statistically significant difference (Kruskal-Wallis test; significance at* $p<0.05$*).*

suggested that a more active application may be beneficial to increasing its bond strength to dentin.[7]

After one year of storage, a significant decrease in bond effectiveness to dentin substrate was observed for all the adhesives tested. Over time, storing the cut beams in water may have accelerated the degradation of the bonding resin or collagen fibrils,[1,28,29] especially for dentin specimens.[30] The presence of water also may have caused swelling and a reduction in the frictional forces between the polymer chains as well as hydrolysis of the filler-matrix interfaces, leading to a decrease in the mechanical properties of the resin.[31,32] Thus, after the long-term storage, mainly an increase of cohesive failure in composite resin and mixed failure of composite resin, adhesive, and dentin were observed for both SBU and CSEB. For ABU, a considerable number of failures between the adhesive and dentin were observed after 24 hours, and the numbers increased after one year of storage. It can be suggested that the growth of the initial defects at the adhesive/dentin interface resulted in the increase of this failure mode pattern in the long term. In addition, smear plugs could be observed in some MTBS-fractured surfaces of ABU. The limited penetration of monomers into the dentin may have weakened the bond performance of this adhesive.

Regardless of the adhesive used, some extent of silver deposits could be detected along the adhesive/dentin interface after thermal aging. In a recent study,[33] a similar leakage pattern was reported at the bottom of the hybrid layer for ABU and SBU after 10,000 thermocycles. Meanwhile, studies have shown that no or little silver particle was observed at the adhesive/dentin interface after 24 hours for ABU, SBU, and CSEB when applied in a self-etch approach to dentin.[6,8,24] The location of silver deposits may indicate areas of the hybrid layer where water remained after evaporating the solvents.[34] Besides MDP, all of the adhesives tested also contain hydroxyethyl methacrylate (HEMA) in their composition. Tay and others[35] have stated that when water is incompletely removed from the primed dentin, porous anionic hydrogels are formed through copolymerization with HEMA and acidic resin monomers. In addition, the presence of water may result in regions of incomplete polymerization in the resin matrix.[35] These regions may allow water permeation, accelerating water sorption, and extraction of unpolymerized or degraded monomers, affecting the durability of the bonding.[35] This might explain the continuous line of silver deposits on the hybrid layer observed in this study.

In contrast, no silver uptake was found for the adhesives tested on etched enamel substrate after 10,000 thermocycles. Resin tags could be observed at the adhesive/enamel interface for all the adhesives using SEM. This finding suggests that the penetration of resin monomers into the etched enamel surface may have encapsulated its crystallite components, providing an effective sealing ability and protecting the outermost enamel from dissolution.[36] There is still a lack of scientific data regarding the sealing performance of ABU, SBU, and ASMP Bond on etched enamel; however, the combined adhesive protocol of CSEB with prior etching of the enamel has been reported to produce scarce leakage in high C-factor cavities *in vitro*,[37] and this protocol has been applied clinically with success regarding marginal integrity and absence of discoloration.[38]

Phosphoric acid etching of enamel prior to adhesive application has been reported to increase its bond performance when compared with the self-etching approach alone.[9,39,40] It is assumed that the use of prior phosphoric acid etching promotes a deeper enamel demineralization, thus increasing the potential for chemical interaction and micro-mechanical interlocking.[41] In this study, CSEB showed significantly higher values in MTBS to etched enamel surfaces than did ABU and SBU after 24 hours, in agreement with findings from a recent study.[12] The CSEB primer penetration into the etched enamel, followed by the application of its bonding agent, may have contributed to the higher initial bond strength values when compared with ABU and SBU, which, as UAs, combine etchant, primer, and the bonding into one application.

On the other hand, after one year of storage, there was a significant decrease within the MTBS values of all materials for etched enamel compared with

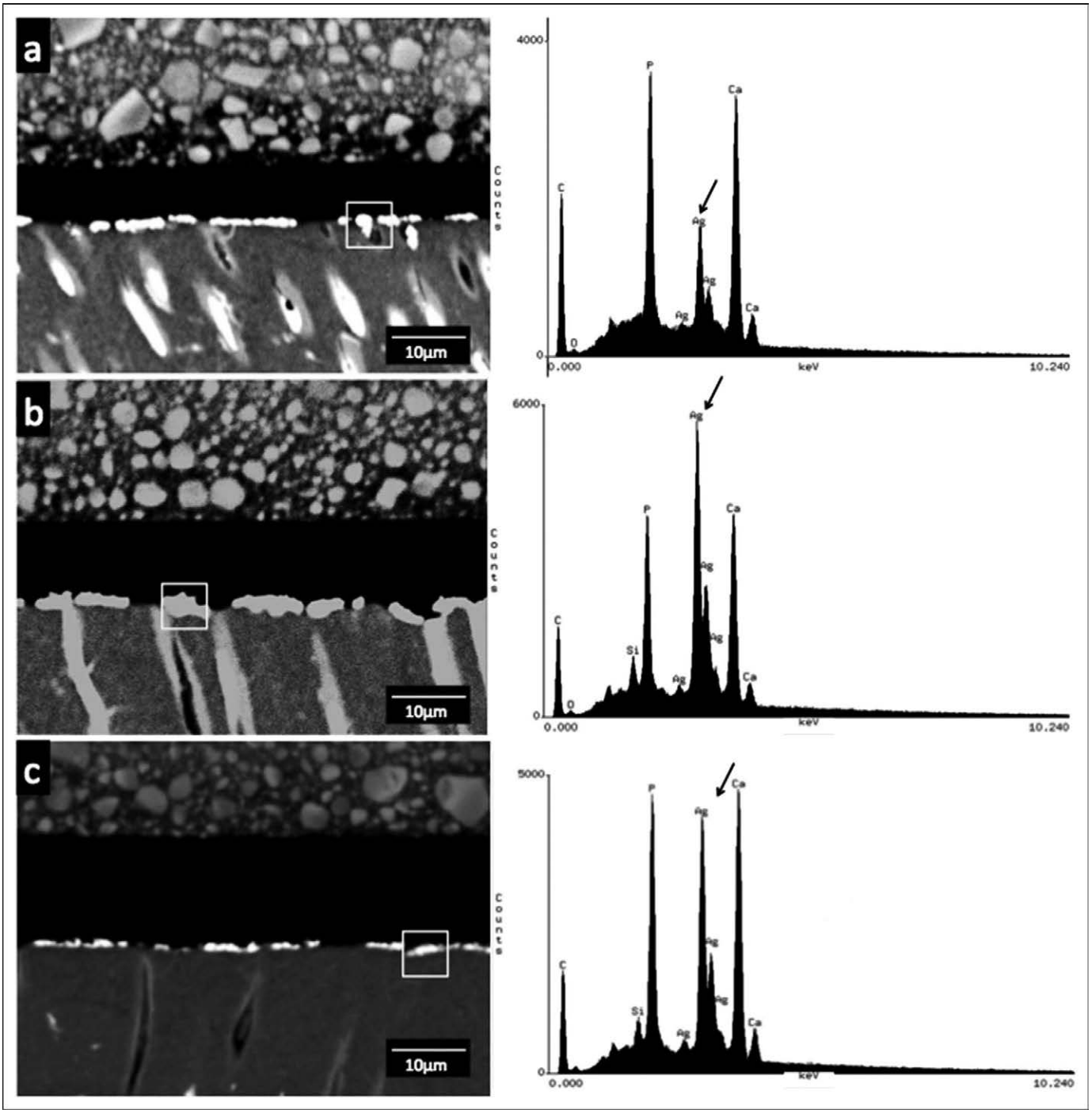

Figure 5. *Energy dispersive x-ray spectroscopy of the same specimens shown in Figure 3. (a): ABU; (b): SBU; and (c): CSEB. A distinct silver peak was observed on the elemental energy spectra (arrow). A map scan (white square) of the same specimen detected metallic silver particles.*

their baseline. It has been reported that the mixture of the hydrophilic monomers contained in the primer with the bonding agent may compromise the durability of the bond.[42-45] In addition, Miyazaki and others[45] observed a small, statistically insignificant decrease in the shear bond strength of self-priming adhesive systems to enamel after 30,000 thermocycles. Conversely, in the current study, storing the MTBS cut beams in water may be considered a form of accelerated aging.[1] Therefore, as with dentin, the collagen fibrils may have degraded and/or the mechanical properties of the resin may have de-

creased after one year when using etched enamel, regardless of the adhesive, because the incidence of cohesive failure in the composite resin increased slightly, as had the frequency of mixed failure in the composite resin, adhesive, and etched enamel.

ASMP Bond is a hydrophobic bonding resin that contains Bis-GMA and HEMA in its formulation. According to the manufacturer,[46] no primer is required when the preparation is in enamel substrate. Therefore, in the current study, the bonding agent was applied directly to a phosphoric acid-etched enamel surface, without the self-etching primer step. As Buonocore showed,[47] bonding to enamel only requires an acid-etch step followed by the application of an unfilled or low-filled hydrophobic resin on air-dried enamel, without the need for an intermediary primer step.[48-50] Because enamel contains very small amounts of water and organics, it has been suggested that the use of hydrophobic resin monomers alone may allow complete infiltration of bonding resin into the demineralized layer.[44,51] By omitting the self-etching primer step of the Adper Scotchbond Multi-Purpose Adhesive system, similar bond strength values were found for ASMP Bond, ABU, and SBU after 24 hours. Compared with the baseline, a significant decrease within MTBS values of ASMP Bond was observed after one year of storage. Although micromechanical interlocking might have contributed to the ASMP Bond results, the absence of MDP monomer as an additional chemical bonding may have accelerated the degradation process. In addition, the storage in water may have decreased the mechanical properties of the polymer matrix,[1] particularly in the thicker adhesive layer of ASMP Bond. It could be related to the increase in the mixed failure of composite resin, adhesive, and etched enamel observed for ASMP Bond after one year of storage.

It is interesting that no significant statistical difference in MTBS values was observed among ABU, SBU, CSEB, and ASMP Bond in etched enamel substrate after one year of storage. Indeed, it seems that the additional chemical bonding to etched enamel can be beneficial to provide bonding stability after long-term storage.[30,52] Takahashi[30] showed that the bond strength to enamel primed with experimental one-step MDP-containing adhesives remained unchanged after 30,000 thermocycles. Nevertheless, the micromechanical interlocking produced by phosphoric acid etching might be essential for effective bond to enamel.[36,53] This could explain the similar MTBS values and the good sealing ability obtained for the MDP-containing adhesives and ASMP Bond after aging.

Enamel is a highly mineralized substrate composed of more than 90 wt% of hydroxyapatite, whereas dentin is a more complex, humid, and porous substrate containing a significant amount of mineral within an organic matrix.[54] This heterogeneous structure and surface morphology likely make dentin less inclined to bond with dental adhesives. Conversely, studies have found that MTBS values of pre-etched enamel surfaces can be similar or even lower than those of dentin surfaces.[55-57] In addition, Sadek and others[55] observed structural defects more frequently on enamel than on dentin specimens before loading, even when lower cutting speeds were used during specimen preparation. It was suggested that cracks propagate more quickly through enamel due to the substrate's brittle and isotropic nature; consequently, the MTBS at the enamel/adhesive interface was lower. With regard to the results of the present study, significantly higher MTBS values were observed using SBU on dentin than on etched enamel substrate; this was true after both 24 hours and one year of storage. In addition, CSEB showed similar initial bond strength regardless of the substrate used, but significantly higher MTBS values were found using dentin after one year. However, using ABU, no significant difference occurred between the two substrates within the same storage period.

Using x-ray diffraction, Yoshihara and others[58] found that MDP can chemically interact with the calcium of hydroxyapatite from dentin and enamel; however, significantly greater chemical reactivity was observed for dentin than enamel. In that same study, the authors suggested that the crystal structure and/or size of hydroxyapatite causes it to be less receptive to chemical interaction within enamel than within dentin. This may partially explain why the bonding effectiveness is lower when using enamel than when using dentin. These findings corroborate the results of the current study when using SBU and CSEB. Although ABU also contains MDP, there was no significant statistical difference between the two substrates, after either storage period. It may be that a more active application of ABU in dentin and enamel substrates increases solvent evaporation, changing the polymer topology by reducing the intrinsic fraction of nanopores and consequently allowing an increase of polymer cross-linking and a degree of conversion inside the dental tissue.[7,59]

In clinical use, UAs can be considered a good alternative with regard to user friendliness and their applicability to different substrates: enamel, dentin, alloys, ceramics, and composites. In a 36-month follow-up clinical report, a UA seemed to maintain good performance when used with non-carious cervical lesions by applying either the etch-and-rinse (wet and dry) strategy to dentin or the self-etch strategy (with or without selective etching) to enamel. However, signs of bonding degradation such as marginal staining were reported when the UA was applied in a self-etch mode without selective enamel etching.[60] That said, applying UAs to dentin using the etch-and-rinse strategy is controversial.[8] Indeed, on the basis of our results, it may be that UAs applied to dentin using a self-etch approach with selective enamel etching is more favorable. Further investigations are required regarding the interaction of UAs with different structures, such as caries-affected dentin and sclerotic dentin because alterations in the mineral content and structure of dentin may compromise the bond,[61] as well as the clinical stability of this promising new category of adhesives.

Within the limitations of this study, the MTBS values revealed statistically significant differences among adhesives and/or storage time for dentin and etched enamel substrates. Therefore, the first null hypothesis has to be rejected. On the other hand, all adhesives showed similar nanoleakage formation along the adhesive/dentin interface, and no silver presence was detected in etched enamel substrate groups after the artificial aging. Thus, the second null hypothesis has to be accepted.

CONCLUSIONS

In the short term, the MTBS values were adhesive and substrate dependent. After aging, the bonding effectiveness of both UAs and the conventional adhesive systems were equal on etched enamel substrate, without leakage; however, UAs may have a bonding performance that is equal or inferior to that of the two-step self-etching adhesive when using dentin as a substrate, with similar amounts of silver deposits at their interfaces.

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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 Piracicaba Dental School, State University of Campinas. The approval code for this study is 143/2014.

Conflict of Interest

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

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Bleaching Effects on Color, Chemical, and Mechanical Properties of White Spot Lesions

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Clinical Relevance

Bleaching initial enamel caries lesions with 10% carbamide peroxide can camouflage white spot lesions without affecting the chemical and mechanical properties of the enamel. Application of casein phosphopeptide–amorphous calcium phosphate is a possible supportive treatment to promote remineralization of caries lesions.

SUMMARY

Objective: The purpose of the study was to evaluate the effect of bleaching on teeth with white spot lesions.

Methods and Materials: Carious lesions with standardized whiteness were produced on the buccal and lingual surfaces of human premolars by pH cycling. Specimens were subjected to four experimental conditions (n=20/group) as follows: group 1, control; group 2, caries formation followed by remineralization using fluoride-containing casein phosphopeptide–amorphous calcium phosphate (CPP-ACP; Tooth Mousse Plus, GC, Tokyo, Japan); group 3, caries formation followed by bleaching using 10% carbamide peroxide; and group 4, caries formation followed by both bleaching and remineralization. The CIE $L^*a^*b^*$ color values were measured with a spectroradiometer, the mineral content was measured with electron probe microanalysis (EPMA) on the cross-sectional surface of each specimen, and the Knoop hardness test was carried out along the EPMA scan line. Two-way analysis of variance was performed with Tukey *post hoc* comparison.

Results: The change in the CIE color values was not significantly different between the caries-formed (ΔE^*=7.03) and the bleached enamel (ΔE^*=7.60). Bleaching of the carious enamel extended the whiteness (ΔE^*=3.38) without additional mineral loss ($p<0.05$). The remineralization treatment significantly increased the calcium (Ca), phosphate (P), and fluoride content of the subsurface lesion area ($p<0.05$). The cross-sectional microhardness

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values correlated well with the Ca and P content (R>0.80).

Conclusions: Bleaching reduced the color disparities between sound and carious enamel without deteriorating the chemical and mechanical properties. The application of CPP-ACP paste enhanced mineral deposition in the subsurface lesion area of carious enamel.

INTRODUCTION

Initial enamel caries causes subsurface demineralization underneath a superficially intact layer. Light is scattered differently on the surfaces of the demineralized enamel compared to the surrounding sound enamel, creating a chalky white appearance.[1] When white spot lesions are exposed to environments enhancing remineralization, additional minerals are adjoined to the superficial layer.[2] With a well-mineralized barrier, ionic ingress into the subsurface body lesion is hampered, resulting in only minimal alteration of the optical characteristics.[3] In clinical settings, the management of white spot lesions often involves removing the lesion and replacing it with a tooth-colored restoration for esthetic improvement. However, this clinical intervention results in a cycle of repair and replacement of the restoration throughout an individual's lifetime.[4]

The ideal management of a white spot lesion would be to enhance its physical appearance and reinforce its weakened substructure in a noninvasive manner. The color changes in demineralized enamel are similar to those created by bleaching procedures, resulting in an increase in lightness and a decrease in yellowness.[5,6] Bleaching of the entire tooth structure containing the white spot lesion may provide a camouflage effect that makes the whiteness of the lesion less visible (Figure 1). However, the application of hydrogen peroxide agents to an already mineral-depleted part of the enamel may bring a potential concern for patients and dental practitioners. Previous studies have reported that bleaching peroxides change the calcium (Ca) and phosphate (P) content of enamel.[7-9] Hence, postbleaching treatment using remineralizing agents has been recommended for restoring the structural integrity of bleached enamel. Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) is known to maintain a supersaturated mineral environment and induce remineralization at the tooth surface by stabilizing high concentrations of Ca and P ions.[10,11] However, clinicians may be uncertain as to whether bleaching teeth with white spot lesions would have acceptable safety and efficacy. In this *in vitro* study, artificial white spot lesions with standardized whiteness were produced on the surface of human premolars. The CIE $L^*a^*b^*$ color values of the specimens were measured with a spectroradiometer at baseline, after the formation of the caries, after bleaching, and after remineralization. Electron probe microanalysis (EPMA) determined the weight percentages of Ca, P, and fluoride (F) in the cross-sectional surface of each specimen. A Knoop microhardness test was carried out along with each EPMA scan line to correlate the mineral content with the cross-sectional hardness of the lesion area. The null hypotheses tested in the study were that 1) bleaching treatment using 10% carbamide peroxide on white spot lesions would not change the color, mineral content, or hardness of enamel and 2) remineralization treatment using CPP-ACP paste would not affect these properties of white spot lesions with or without bleaching.

METHODS AND MATERIALS

Specimen Preparation

Twenty human upper premolars extracted during dental treatments were used under the approval of the local Institutional Review Board. The teeth were disinfected in 0.5% chloramine-T for one week, stored in distilled water at 4°C, and then inspected under a 10× stereomicroscope (Jaemyung Ind, Seoul, Korea) to ensure that there were no white spot lesions or other defects. The roots of the teeth were removed at the cementoenamel junction with a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). The crown part was sectioned mesiodistally and buccolingually into four parts. Each quarter of the crown was ground on the dentin side, leaving a 2-mm-thick dentin layer, and was randomly distributed into four experimental groups (Figure 2). The sections were then embedded in acrylic resin with a 2×4 mm window on the exposed enamel surface.

Artificial Caries Lesion Formation

To form the artificial caries lesions, pH cycling was applied three-times (12 days). Each specimen was immersed in 2.5 mL of demineralizing solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50 mM acetate buffer, pH 4.8) for 72 hours, followed by immersion in 2.5 mL of remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES], pH 7.0) for 24 hours at 4°C with daily changes of the solution.

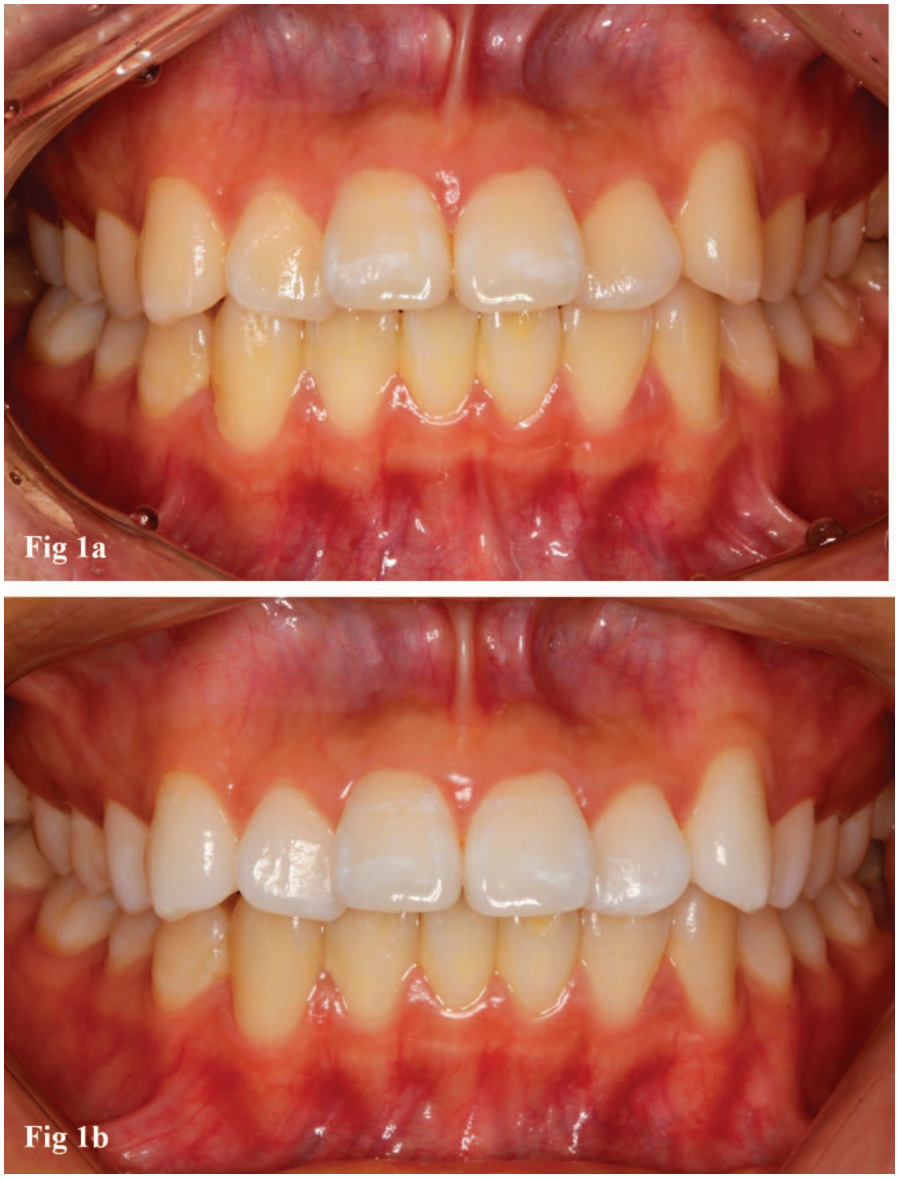


Figure 1. *(a) Long-existing white spot lesions on the maxillary central incisors have a well-mineralized and hardened enamel surface that does not require a restorative treatment. (b) Bleaching enhanced overall whiteness in the upper dentition. White spot lesions became less noticeable after bleaching.*

Bleaching and Remineralization Treatment

For the bleaching treatment, 10% carbamide peroxide gel (Opalescence Non-PF 10%, Ultradent, South Jordan, UT, USA) was applied on the exposed enamel surfaces with the aid of a plastic mold to create a 1 mm thickness (Sof-Tray Classic Sheets, Ultradent) and maintained for eight hours. The specimens were washed with distilled water to remove the residual carbamide peroxide gel after bleaching and stored in artificial saliva for 16 hours. This daily bleaching procedure was repeated for 14 days. The pH of the bleaching gel used in the experiment was measured as 6.8.

During the remineralization procedure, F-containing CPP-ACP paste (Tooth Mousse Plus, GC, Tokyo, Japan) was applied on the enamel surface for 30 minutes twice a day for 14 days. After the completion of each application, the specimens were washed with distilled water and stored in artificial saliva. For the specimens in group 4, CPP-ACP paste was applied to the specimen shortly after the bleaching gel was washed off.

CIE *L*a*b** Color Measurement

The color of the enamel surface was measured at baseline, after formation of the white spot lesion, and after completion of bleaching and/or remineralization. For the color measurement, the specimens were retrieved from storage solution, dried with blotting paper, and immediately placed in a light booth (Color

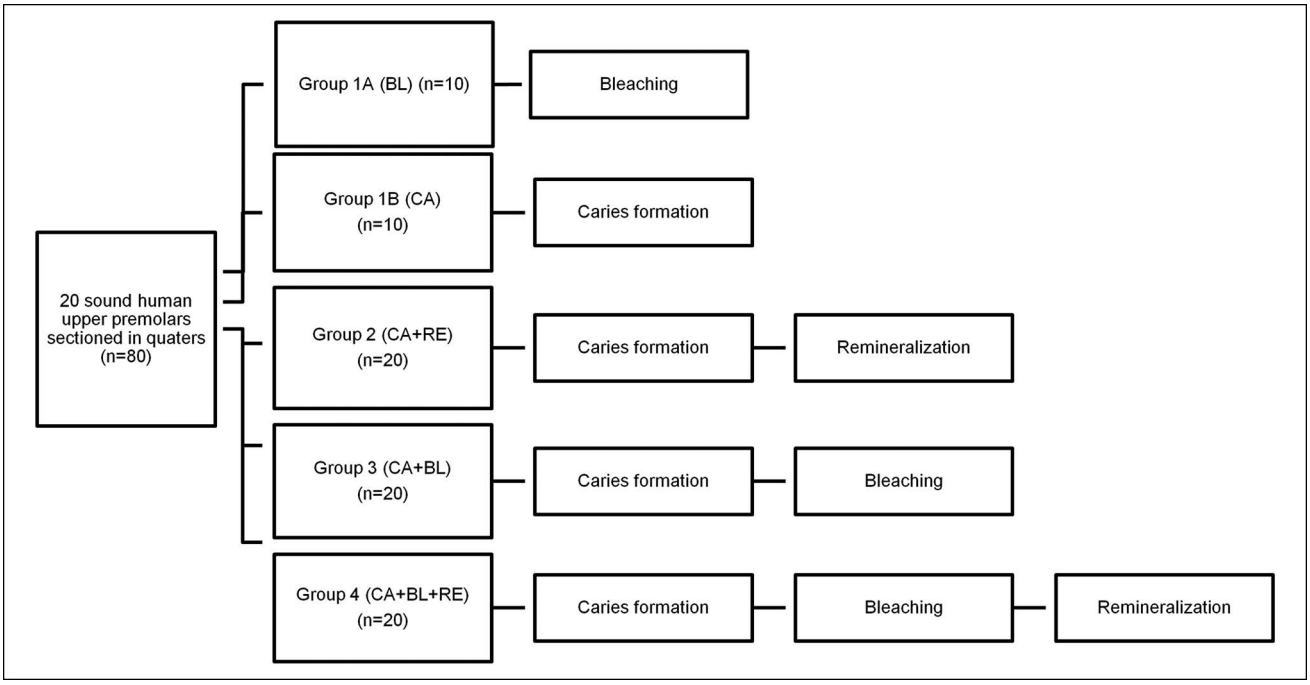


Figure 2. *Procedural steps in four experimental groups.*

Sense II; Sungjin Hitech, Gyeonggi-Do, Korea) with Munsell N7 neutral gray walls and floor.

A spectroradiometer (PR-670; SpectraScan, Photo Research, Chatsworth, CA, USA) equipped with a Macro-Spectar MS-75 lens (Photo Research) was fixed on a tripod at a distance of 355 mm from the measured object and with a measurement area of 2.63 mm in diameter, providing an optical configuration of 2° observation to the object. Four D65 simulating tubes (F2DT12/65; Gretagmacbeth, Research Triangle Park, NC, USA), reportedly having a correlated color temperature of 6500 K and a color rendering index of 91, were used as the light source. The tubes were bidirectionally fixed with a 45° illumination angle at a distance of 30 cm from the measured object. External light was excluded by covering the equipment with a light-proof cover. The positioning of the lens toward the surface of the specimen was kept constant to ensure a standardized measurement throughout the experiment. Spectral reflectance was obtained from 380 to 780 nm with a 2-nm interval (Spectrawin 2.0, Photo Research) and was subsequently converted to CIE L^*, a^*, and b^* values. The color difference (ΔE^*) was calculated by the equation: $(\Delta E^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Every measurement was performed after calibration over a white background and repeated three times.

EPMA

Specimens were embedded in epoxy resin (Epofix, Struers, Glasgow, UK) and cross-sectioned along the midline. The cut surfaces were serially polished with 1200-, 2400-, and 4000-grit silicon carbide abrasive papers, followed by 1-μm and 0.25-μm diamond and 0.1-μm and 0.05-μm aluminum oxide polishing suspensions (Struers, Copenhagen, Denmark). The specimens were ultrasonically cleaned in deionized water for 10 minutes, dried for 72 hours in a desiccator, and then sputter-coated with carbon. The demineralization area on the cross-sectioned enamel surfaces was identified using the phase contrast of the backscattered electron imaging mode of a scanning electron microscope (SEM) (JEOL JSM-6610LV, JEOL, Akishima, Japan). Two-line analyses were performed perpendicular to the outer enamel surface at 0.3-μm pixel intervals. The observation areas (intact surface layer, demineralized subsurface layer, and inner sound enamel) were determined according to changes in Ca and P content using an electron microprobe (JEOL JXA-8100, JEOL, Akishima, Japan). The operating conditions for the elemental analyses were 15 kV of accelerating voltage and 50 nA of beam current. A fluorapatite crystal (3.38% F) was used as a standard comparison for analysis.

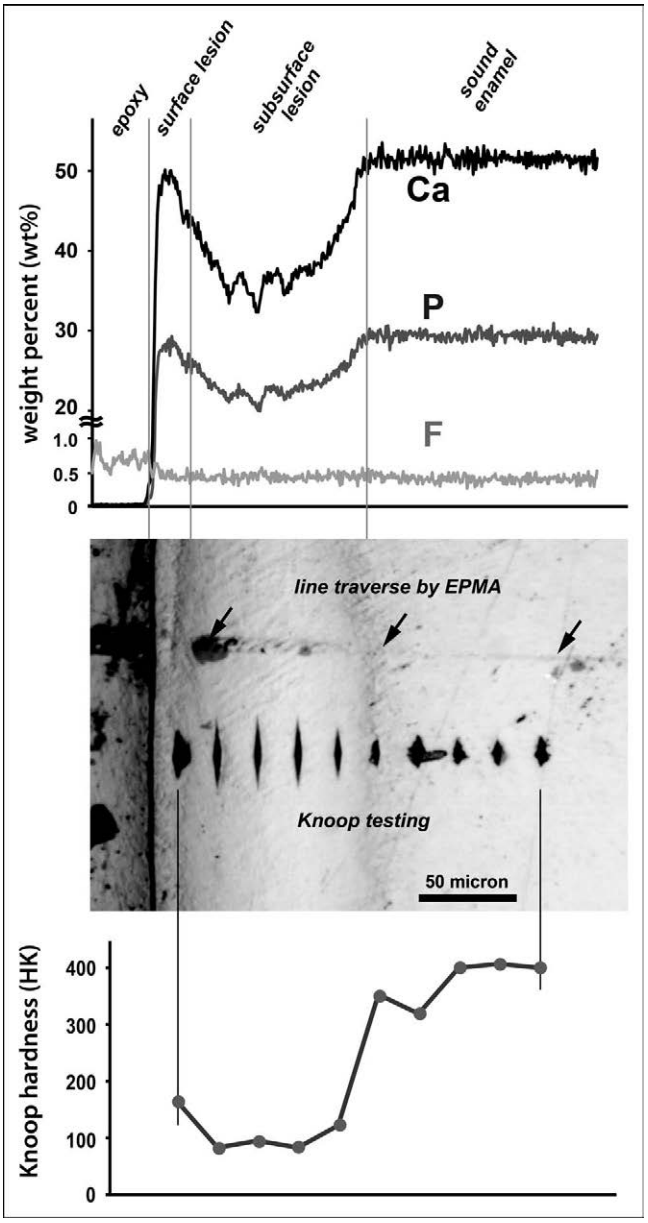


Figure 3. *Elemental analysis along the scan line (dark arrows) illustrated the elemental composition (Ca, P, and F) on the cross-sectional SEM image. Knoop microhardness values measured along the scan line corresponded to the elemental contents of each indentation site.*

Knoop Microhardness Measurement

Cross-sectional enamel surface hardness was measured using a microhardness tester (Tukon 2100, Instron Corp, Canton, MA, USA), and a Knoop diamond indenter with a load of 10*g* was applied for 11.5 seconds. A total of 10 indentations were made with a 20-μm interval from the surface to the sound enamel alongside an EPMA scan line (Figure 3).

Statistical Analysis

The sample size calculation was based on the data from a pilot study using the microhardness test and had 80% power to detect a 30–standard deviation (SD) difference between any two groups, assuming an overall 5% significance level and two-sided tests. The normality and homogeneity of the samples were tested using the Kolmogorov-Smirnov test. Assuming a normal distribution of differences, two-way analysis of variance was performed with Tukey *post hoc* comparison. The mean values of CIE L^*, a^*, and b^* and the differences between each measurement point were compared among the four groups. The mean percentage weight loss of Ca and P and the mean weight of F in the surface and the subsurface layer were compared among the groups. The mean values and percentage decreases of the microhardness at the lesion areas were compared among the groups. The correlations between the Ca and P content and microhardness values were evaluated by Pearson correlation coefficients. A p-value of 0.05 was selected as the threshold for statistical significance. Analyses were performed using SPSS 13.0 (SPSS, Chicago, IL, USA).

RESULTS

The CIE $L^*a^*b^*$ values of the specimens at baseline (BA) and after caries formation were not significantly different among all groups. The final color change after treatment of bleaching, remineralization (RE) and/or bleaching (BL), $\Delta E^*_{\text{TR-BA}}$, ranged from 7.03 to 7.60 in groups 1A (BL), 1B (CA), and 2 (CA+RE), without any significant differences (Table 1). However, the $\Delta E^*_{\text{TR-BA}}$ values in group 3 (CA+BL) and group 4 (CA+BL+RE) were 10.98 and 10.81, respectively, which was significantly greater than those in the other three groups ($p<0.05$). The differences in the three color parameters, $\Delta L^*_{\text{TR-BA}}$, $\Delta a^*_{\text{TR-BA}}$, and $\Delta b^*_{\text{TR-BA}}$, were in accordance with $\Delta E^*_{\text{TR-BA}}$; each value in groups 3 (CA+RE) and 4 (CA+BL+RE) was significantly larger than its counterpart in the other three groups ($p<0.05$).

The mean (SD) depth of the surface and subsurface lesion areas ranged from 23.4 (5.8) μm to 157.8 (19.0) μm, respectively, without any significant difference among all groups (Table 2). The amount of Ca and P loss in the subsurface lesion in group 2 (CA+RE) was significantly less than that in group 1B (CA) ($p<0.05$). The mean (SD) Ca/P ratios were 2.17 (0.10) and 2.15 (0.01) in the surface and subsurface lesion areas, respectively, without significant differences among groups. The weight of F was highest in the surface lesion, followed by the

Table 1: *Comparison of the Mean (SD) Values of Color Difference Between at Baseline (BA) and After Treatment (TR) Among the Experimental Groups*

Group: Treatment	N	Color Difference Between at Basement and After Treatment[a]			
		$\Delta L^*_{(TR-BA)}$	$\Delta a^*_{(TR-BA)}$	$\Delta b^*_{(TR-BA)}$	$\Delta E^*_{(TR-BA)}$
1A (BL)	10	4.95 (0.55) A	−1.13 (0.15) A	−5.60 (1.03) A	7.60 (0.84) A
1B (CA)	10	4.72 (0.54) A	−0.50 (0.12) A	−5.14 (0.95) A	7.03 (0.90) A
2 (CA + RE)	20	4.69 (0.75) A	−0.46 (0.13) B	−5.30 (1.14) A	7.14 (1.09) A
3 (CA + BL)	20	6.96 (0.75) B	−1.47 (0.24) C	−8.31 (0.94) B	10.98 (0.80) B
4 (CA + BL + RE)	20	6.86 (0.69) B	−1.40 (0.23) C	−8.20 (0.86) B	10.81 (0.85) B

Abbreviations: BL, bleaching; CA, caries formation; RE, remineralization.
[a] *Different superscript letters following means denote significant differences (*$p<0.05$*).*

subsurface lesion and the inner sound enamel across all groups ($p<0.05$, Figure 4). The amount of F in the subsurface lesion was higher in group 2 (CA+RE) than in group 1B (CA) ($p<0.05$).

The mean (SD) Knoop microhardness value was 231.3 (99.3) H_k in the surface lesion, 324.5 (76.0) H_k in the subsurface lesion, and 426 (38.7) H_k in the sound enamel (Table 3). Each microhardness value of the surface, subsurface, and total lesion area did not show a significant difference across groups (Figure 5). A strong correlation existed between the Ca and P content and the microhardness values for the subsurface lesion area (Figure 6).

DISCUSSION

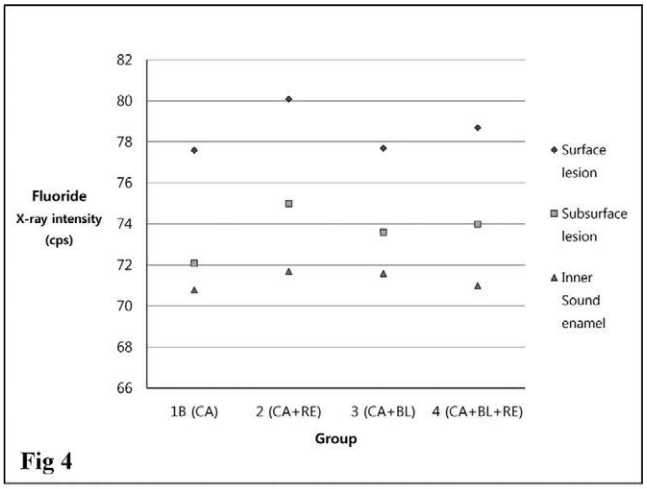
For our first hypothesis, we primarily investigated whether the whiteness of carious enamel could be masked by the whitening effect of the surrounding sound enamel structure. The color change produced by the formation of artificial caries was similar to that obtained from peroxide bleaching. The increase in lightness (L^*) and decrease in yellowness (b^*) contributed to the whitening of artificial caries, which was in accordance with the results of previous studies.[5,6] The color of artificially formed carious enamel ($\Delta E^*=7.03$) was further changed by bleaching, resulting in extended whiteness ($\Delta E^*=10.98$). However, considering the outcome of bleaching sound enamel ($\Delta E^*=7.60$), the color discrepancy between the sound and carious enamel after bleaching (3.38 ΔE^* units) was within a relatively acceptable range. In a widely cited study by Johnston and Kao,[12] 3.7 ΔE^* units was proposed as the perceptibility threshold and 6.8 ΔE^* units was proposed to be the borderline for color mismatch. Therefore, from an esthetic standpoint, the bleaching treatment of teeth containing white spot lesions may be a clinically relevant procedure to promote an optical camouflage effect.

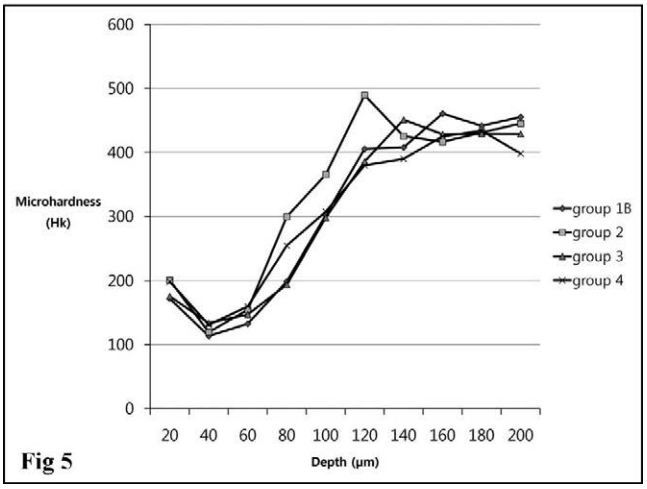
Our second question was whether bleaching treatment would cause further demineralization of the white spot lesion. We created a superficially intact enamel layer with a relatively uniform width of 22-24 μm, in which the loss of Ca and P was minimal (0-2 wt%). The underlying subsurface area with mineral loss (15-25 wt%) had a depth of 150-160 μm. This mineral-depleted porous substructure induced an altered light-scattering mode within the enamel structure, producing a whitish appearance. Many previous studies have reported on the potential impact of bleaching on the enamel microstructure; those results were influenced by many variables, such as tooth type, peroxide concentration, the pH of the bleaching agent, the duration of contact, and the treatment interval. In this study,

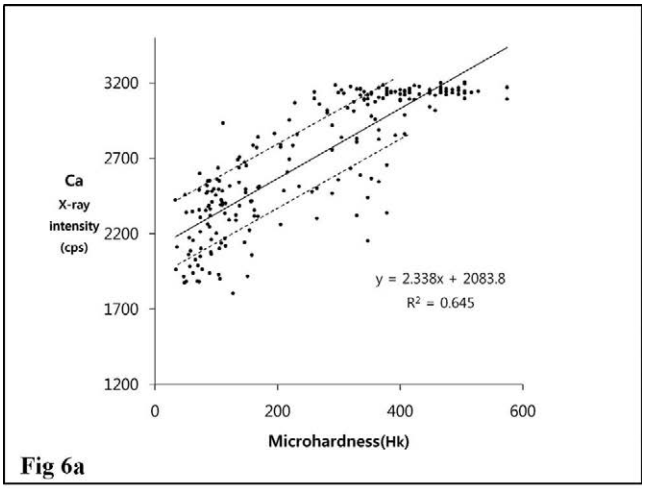
Table 2: *The Mean (SD) Depth (μm) and Weight Loss (%) of Calcium (Ca) and Phosphorus (P) on the Surface and the Subsurface Lesions*[a]

Group (Treatment)	N	Surface Lesion			Subsurface Lesion		
		Depth, μm	Ca Loss, wt%	P Loss, wt%	Depth, μm	Ca Loss, wt%	P Loss, wt%
1B (CA)	10	24.2 (7.9)	2.6 (4.8)	1.5 (5.6)	164.2 (20.7)	25.2 (8.5) A	24.8 (8.5) A
2 (CA+RE)	10	23.9 (6.6)	0 (3.8)	1.4 (3.7)	153.7 (12.5)	16.2 (5.6) B	15.5 (6.0) B
3 (CA+BL)	10	22.9 (5.4)	2.1 (4.8)	1.9 (3.0)	165.7 (18.4)	21 (7.2) AB	21.3 (7.1) AB
4 (CA+BL+RE)	10	22.3 (6.2)	0.2 (1.9)	1.5 (1.1)	157.4 (24.8)	19.8 (6.7) AB	18.4 (5.7) AB

Abbreviations: BL, bleaching; Ca, calcium; CA, caries formation; P, phosphorus; RE, remineralization.
[a] *Different superscript letters following means denote significant differences at* $p < 0.05$.







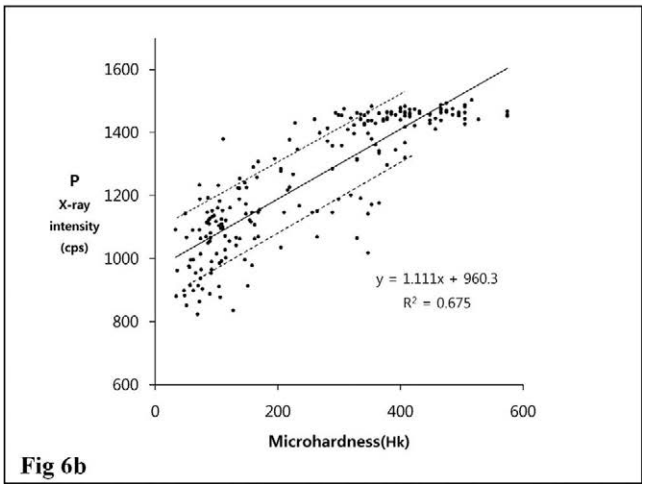


Figure 4. *The mean content of F in each lesion area.*
Figure 5. *The mean cross-sectional Knoop microhardness values of specimens from the four experimental groups. The Knoop microhardness values were measured at intervals of 20 μm starting from the surface enamel layer and reaching to the 200-μm-deep sound enamel.*

Figure 6. *Correlation coefficients and regression equations between Ca and P contents and microhardness values in the subsurface lesion area of the carious enamel.*

bleaching using 10% carbamide peroxide with a pH of slightly less than 7 did not induce a significant mineral loss in carious enamel. We obtained the cross-sectional microhardness values at each location adjacent to the EPMA scan line in order to evaluate the depth-related chemical and mechanical properties. White spot lesions represent the preliminary stage of subsurface enamel demineralization, although they do not necessitate invasive restorative intervention. Hence, it is clinically meaningful to reharden the surface enamel and maintain its mechanical integrity. The Ca and P content and

Table 3: *The Mean (SD) Microhardness and Percentile Decrease of the Surface and Subsurface Lesions*

Group (Treatment)	N	Surface Lesion		Subsurface Lesion		Total Lesion	
		Microhardness, H_k[a]	Decrement, %[b]	Microhardness, H_k	Decrement, %	Microhardness, H_k	Decrement, %
1B (CA)	10	172.2 (49.6)	59.9 (16.1)	288.7 (47.8)	34.6 (13.6)	274.1 (44.8)	37.7 (13.6)
2 (CA+RE)	10	201.0 (27.4)	53.7 (8.1)	324.3 (46.2)	25.8 (9.4)	308.9 (39.7)	29.3 (8.2)
3 (CA+BL)	10	175.8 (19.8)	58.6 (5.7)	291.1 (86.9)	32.7 (16.6)	276.7 (77.6)	35.9 (14.8)
4 (CA+BL+RE)	10	198.6 (18.1)	51.9 (5.8)	292.4 (47.9)	29.2 (12.8)	280.7 (41.7)	32.1 (11.3)

Abbreviations: BL, bleaching; CA, caries formation; RE, remineralization.
[a] $H_k = 14.12P/\ell^2$ (kgf/mm^2).
[b] *Decrement (%) = (1−the mean microhardness value of each lesion area/the mean of the inner sound enamel) × 100.*

hardness values were relatively well correlated throughout the lesion area. Only a small disparity existed at the first 20-μm surface area with decreased hardness. The surface of the enamel with the lesion was softened and porous but mineral-abundant.[13] This mechanically weakened layer may be easily abraded by normal tooth brushing. In a clinical evaluation[14] of initial caries, the remission of whiteness was not entirely due to color reversal from the remineralized microstructure but rather was due to mechanical removal of the superficially weakened layer. The hardness of the cross-sectional enamel gradually increased, with elevating mineral content reaching the level of the sound enamel. Overall, bleaching using 10% carbamide peroxide did not deteriorate the chemical and mechanical properties of carious enamel. Our first hypothesis was accepted.

To test our second hypothesis, we evaluated the effect of CPP-ACP on color, mineralization, and hardness of carious enamel before and after bleaching. Despite confirmation of mineral gain in the subsurface lesion, no significant color reversal was detected by the spectroradiometer. This corresponds to a common clinical situation, with long-existing caries lesions arrested or regressed by well-mineralized surface enamel but still showing a whitish appearance. Even when some mineral deposition occurs in the underlying body lesion, the pore volume is decreased but the pore number is unchanged.[2] There are several stages of mineral deposition, including the formation and growth of new crystals and the regrowth of preexisting crystals.[15] Although the Ca/P ratio remains unchanged, the heterogeneity of the modified apatite structure contributes to the altered optical characteristics.[16] The color mismatch between the remineralized and sound enamel substrate is unsolved, often requiring esthetic enhancement.

As for the remineralizing and rehardening effect of CPP-ACP on enamel during and after at-home bleaching procedures, many studies suffered from dissimilar experimental conditions; some studies used bovine teeth, which are more porous than human teeth,[10,17] while others measured the surface microhardness or roughness on the superficial enamel instead of its subsurface structure.[11,18,19] In this study, the change in mineralization was evaluated both at the surface and subsurface lesion areas after the use of CPP-ACP. We determined that carious enamel without bleaching had the largest remineralization gains. In group 2, freshly formed carious lesions, which were not subjected to a sequence of 10% carbamide application and artificial saliva storage, largely promoted incorporation of free ions from the CPP-ACP paste into the subsurface lesion area. The microhardness values were also highest in group 2, both at the surface and subsurface lesion areas, and the values in other groups followed the same order as in the mineral composition. Overall, our second hypothesis was not accepted.

Many previous studies[9,20,21] have determined the effect of remineralizing agents on bleached teeth and concluded that any substantial recovery of hardness or mineral deposition was mainly due to supplementary ions in the storage media (artificial or human saliva). Under *in vivo* conditions, the repair mechanism would more actively counteract the mineral loss than under *in vitro* conditions, even in the case that the bleaching treatment might cause an initial deterioration of the chemical or mechanical properties of the enamel. We confirmed that the application of CPP-ACP paste enhanced the reversal of the early caries lesion stage, as shown in other studies.[3,22] Considering the largest change shown in group 2, CPP-ACP's remineralizing effect on white spot lesions seemed to be maximized prior to the bleaching procedure.

CONCLUSIONS

In this study, the 10% carbamide peroxide bleaching of enamel with white spot lesions decreased color disparities without deteriorating mineral composition or microhardness. The application of CPP-ACP paste promoted mineral gain in the subsurface body lesion. The bleaching treatment for teeth with white spot lesions can be recommended as a noninvasive esthetic treatment regimen with supplementary remineralization protocols.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Seoul National University Dental Hospital Institutional Review Board. The approval code for this study is CRI13010.

Conflict of Interest

The authors 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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Simulated Wear of Self-Adhesive Resin Cements

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Clinical Relevance

Loss of cement at the margins of restorations can initiate a variety of clinical issues that may ultimately result in restoration loss and replacement.

SUMMARY

One of the primary areas of concern with luting agents is marginal gap erosion and attrition. The purpose of this laboratory study was to evaluate bulk and marginal slit (gap) generalized wear of self-adhesive resin cements. Three self-adhesive resin cements were used in this study: G-CEM LinkAce (LA), Maxcem Elite (ME), and RelyX Unicem2 Automix (RU). A custom stainless-steel fixture with a cavity 4.5 mm in diameter and 4 mm deep was used for simulated generalized (bulk) wear. For simulated marginal gap wear, a two-piece stainless-steel custom fixture was designed with a slit (gap) 300 μm wide and 3 mm in length. For both wear models, 20 specimens each for each of the three adhesive cements were made for both light-cure and chemical-cure techniques. The cured cements were polished with a series of carbide papers to a 4000-grit surface and subjected to 100,000 cycles using the slit (gap) wear model and 400,000 cycles for generalized (bulk) wear in a Leinfelder-Suzuki (Alabama machine) wear simulator (maximum load of 78.5 N). Flat-ended stainless-steel antagonists were used in a water slurry of poly(methylmethacrylate) beads for simulation of generalized contact-free area wear with both wear models. Before and after the wear challenges, the specimens were profiled with a Proscan 2100 noncontact profilometer, and wear (volume loss [VL] and mean facet depth [FD]) was determined using AnSur 3D software. Two-way analysis of variance (ANOVA) and Tukey *post hoc* tests were used for data analysis for the two wear models. Scanning electron microscopy (SEM) was used to examine polished surfaces of the resin cements and the worn surfaces after the wear

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challenges. The two-way ANOVA of VL using the generalized (bulk) wear model showed a significant effect among the three resin cement materials for the factor of resin cement ($p<0.001$) and the interaction of the cement and cure method ($p<0.001$), but not for the cure method ($p=0.465$). The two-way ANOVA for FD also found a significant difference for the factor of resin cement ($p<0.001$) and the interaction of the resin cement and cure method ($p<0.001$), but not for the cure method ($p=0.277$). The simulated generalized (bulk) wear for the light-cure groups was as follows: VL (mm^3): RU 0.631 (0.094), LA 0.692 (0.112), and ME 1.046 (0.141) and FD (μm): RU 43.6 (6.5), LA 47.0 (7.7), and ME 72.5 (9.9). The simulated generalized (bulk) wear for the chemical-cure groups was as follows: VL (mm^3): LA 0.741 (0.105), RU 1.231 (0.234), and ME 1.305 (0.143) and FD (μm): LA 50.7 (7.2), RU 84.5 (16.1), and ME 91.7 (10.2). Simulated wear using the slit (gap) model for the light-cure groups was as follows: VL (mm^3): RU 0.030 (0.006), LA 0.031 (0.006), and ME 0.041 (0.009) and FD (μm): RU 49.6 (5.7), LA 57.2 (8.4), and ME 70.9 (10.7). The wear values for the chemical-cure slit (gap) groups were as follows: VL (mm^3): LA 0.031 (0.004), ME 0.038 (0.007), and RU 0.045 (0.009) and FD (μm): LA 53.9 (6.7), ME 63.5 (9.1), and RU 74.2 (12.9). Pearson correlation tests revealed a strong relationship between the two wear models for the light-cure groups and a good relationship for the chemical-cure groups. The observations using SEM showed differences in filler particle shape and size among the cements and the resultant effect of the wear challenges. The worn surfaces of each cement were essentially the same for both light-cure and chemical-cure methods. The bulk wear model and new slit (gap) model for evaluation of simulated generalized wear of luting agents demonstrated significant differences ($p<0.05$) in relative wear among three self-adhesive resin cements and between visible light- and chemical-cure techniques.

INTRODUCTION

The evolution of adhesive dentistry procedures and materials has changed many facets of dentistry. The development of resin luting agents, along with adhesive dentistry techniques, has rapidly advanced the capability to bond indirect restorations to mineralized tooth structures and core buildup materials. One of the main advantages of resin cements, when compared to nonpolymer luting agents, is enhanced mechanical properties and the ability to adhesively bond to metal, ceramic, enamel, and dentin.[1-8] The use of etch-and-rinse bonding procedures along with resin luting agents has helped to promote the use of high-strength ceramic restorations. The more recent introduction of self-adhesive resin cements has reduced the required number of clinical steps in the bonding sequence, thereby reducing the number of treatment steps along with patient chair time. The dual curing capability (light-cure and chemical-cure) of many of the newer resin cements has extended their use to include restorations where light penetration to the intaglio surface is limited or not attainable. The use of resin cements is now also well established for fiber-reinforced composite materials and has extended into orthodontics for attachment of both metallic and ceramic brackets.

One of the challenges that remains, regardless of the cementing media, is marginal integrity.[9] A major concern with all dental cements is their ability to resist gap formation at the marginal closure area from attrition and erosion. Intact restoration margins reduce the potential for marginal staining, secondary caries, tooth sensitivity, and periodontal issues. Clinical criteria have been used to assess marginal integrity in long-term clinical trials. However, these studies are costly and take years to complete. Investigators have used laboratory marginal gap studies in an effort to assess the potential for cement loss at the margins of restorations.[9-12] These studies have shown an excellent relationship among 1) tooth margin and restoration gap width, 2) type of cement, and 3) cement wear. They found that enhanced wear resistance of resin cements is associated with a smaller filler particle size.

Depending on the clinical situation, dual-cure self-adhesive resin cements can be light-cured, chemically-cured, or can use a combination of light curing and chemical curing. The degree of polymerization conversion can also impact the wear resistance of a cement at the margin.[13-15] Less efficiency in polymerization is often seen in chemically-cured resin cements and may lead to higher wear.[15] Several self-adhesive resin cements are now available to the profession. The acidic monomers in these formulations can be a challenge when designing chemical-cure based polymerization components, as many amine initiators are quenched at low pH, leading to a lower degree of polymerization when compared to light curing.[15-17]

Table 1: *Self-Adhesive Resin Cements*

Material	Manufacturer	Lot	Shade	Study Code
G-CEM Link Ace	GC Corp (Tokyo, Japan)	1212144	A2	LA
Maxcem Elite	Kerr Corp (Orange, CA, USA)	4818000	Brown	ME
RelyX Unicem2 Automix	3M ESPE Dental Products (St Paul, MN, USA)	50153	A2	RU

Technology has rapidly advanced for assessing simulated laboratory wear by the introduction of noncontact optical profilometers and enhanced computer software for data analysis.[18] In addition, a new marginal slit (gap) model was developed for laboratory-generalized wear (contact-free area [CFA] wear) simulation in an effort to learn more about the wear resistance of newer-generation self-adhesive resin cements. The purpose of this study was to use the newly developed slit (gap) wear model and an established generalized (bulk) wear model to assess relative wear characteristics of newer dual-cure self-adhesive resin cements. The two hypotheses tested were the null hypotheses that 1) there will not be a significant difference (α=0.05) in wear values among three dual-cured self-adhesive resin cements and that 2) wear of self-adhesive resin cements using light-cure or chemical-cure methods will not be different.

METHODS AND MATERIALS

Three self-adhesive resin cements (Table 1) were evaluated in this study: G-CEM LinkAce (LA) (GC Corp, Tokyo, Japan), Maxcem Elite (ME) (Kerr Corp, Orange, CA, USA), and RelyX Unicem2 Automix (RU) (3M ESPE Dental Products, St Paul, MN, USA). The evaluation components in this study included 1) simulated generalized (bulk) wear testing, 2) simulated marginal slit (gap) wear testing, 3) argon-ion etching scanning electron microscopy (SEM) of the resin cement surfaces, and 4) SEM examination of the cement wear facets.

Generalized (Bulk) Wear Simulation

Forty specimens for each of the three self-etching resin cement materials (total of 120 specimens) were prepared for wear challenges of 400,000 cycles using a generalized (bulk) wear model (CFA wear) in a Leinfelder-Suzuki (Alabama) wear simulation device. The methodology for sample preparation and the generalized wear model was previously described by Barkmeier and others.[18-20] In summary, stainless-steel custom fixtures with cavities 4.5 mm in diameter and 4 mm deep were used to hold the resin cement materials. Twenty specimens for each resin cement were light-cured in two increments of approximately 2 mm for 40 seconds with a Spectrum 800 curing unit (Dentsply Caulk, Milford, DE, USA) set at 600 mW/cm^2. The other 20 specimens for each cement) were chemically-cured (the light curing unit was not used). After 24 hours, the cement surfaces were polished flat to 4,000 grit (Figure 1) using a sequence of silicon carbide papers (Struers Inc, Cleveland, OH, USA).

The custom fixtures were mounted inside a plastic water bath, and a brass cylinder was placed around each fixture. The water bath fixture was then attached to the wear simulator. A water slurry of poly(methylmethacrylate) (PMMA) was used as the abrasive media and placed inside the cylinders over the resin cement specimens.

Stainless-steel antagonists 6.5 mm in diameter (Figure 2), mounted in spring-loaded pistons, were then used to deliver the wear challenges in the wear simulation machine (Figure 3). The pistons rotated approximately 30 degrees as the load was applied (maximum load of 78.5 N) at a rate of 2 Hz and then

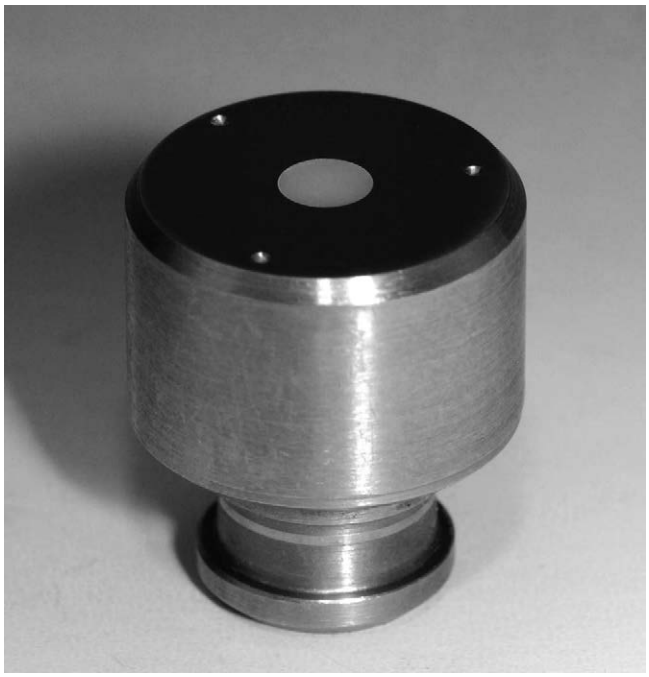


Figure 1. *Custom stainless-steel fixture for generalized (bulk) wear.*

Figure 2. *Stainless-steel flat-ended antagonist.*

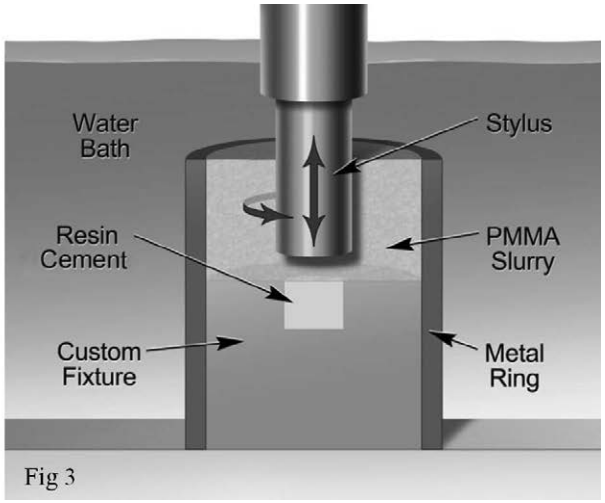


Figure 3. *Generalized wear model.*

counterrotated back to their original position as the cycle was completed.

Prior to wear testing, the specimens for each resin composite material were profiled using a Proscan 2100 noncontact optical profilometer (Scantron Industrial Products, Ltd, Taunton, UK) with Proscan software. The individual scanned surfaces were used as the pretest digitized surface contour for each specimen.

Following the 400,000 cycling period, the specimens were ultrasonically cleaned (L&R Solid State Ultrasonic T-14B, South Orange, NJ, USA) for 3 minutes in distilled water and then again profiled using the Proscan 2100 unit. The X, Y, and Z coordinates of the before and after scans from the Proscan software were exported for analysis with AnSur 3D software (Minnesota Dental Research Center for Biomaterials and Biomechanics, University of Minnesota, Minneapolis, MN, USA).

Wear measurements were determined from differences between the before and after data sets. A computerized fit was accomplished on the before and after surface contours using AnSur 3D. Volume loss (VL) (mm^3) and facet depth (FD) (μm) of the wear areas were recorded for each generalized wear specimen.

Slit (Gap) Model: Generalized Wear Simulation

A two-piece stainless-steel custom fixture was designed to examine resin cement wear using a thin slit or gap. This design was used in an attempt to simulate generalized wear (CFA wear) of resin cements at the marginal closure area. The overall fixture size was the same as the stainless-steel custom fixture used for generalized (bulk) wear, but a thin slit replaced the cylindrical cavity in the center of the top flat surface. The two-piece fixture was designed to have a slit (gap) 300 μm wide, 3 mm in length, and 4 mm in depth (Figures 4 and 5). A 3% paraffin-in-hexane solution was used as a separating media on the interior walls of the two-piece fixture.

Twenty specimens each of the three self-adhesive resin cements were made for both light-cure and chemical-cure groups. The cured cements were polished and subjected to 100,000 cycles in the same manner as described above for generalized (bulk)

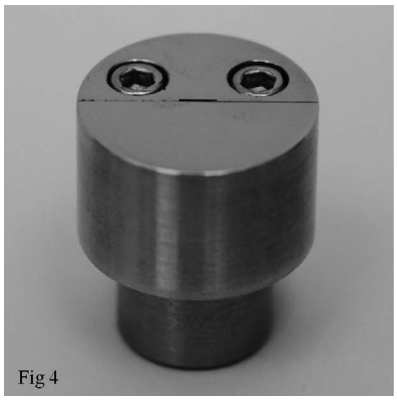


Figure 4. *Slit (gap) fixture for generalized wear simulation.*

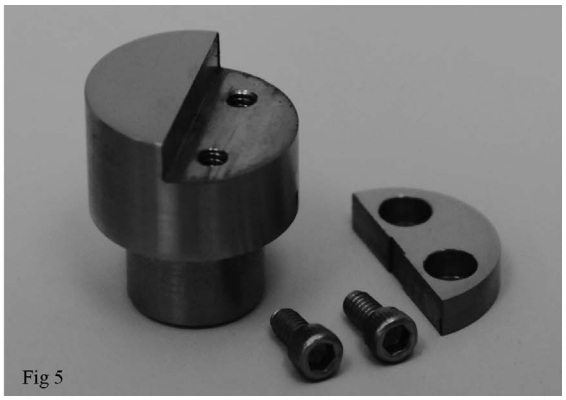


Figure 5. *Unassembled slit (gap) fixture.*

Table 2: *Two-Way Analysis of Variance: Simulated Generalized (Bulk) Wear, Volume Loss*

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F*-Ratio	*p*
Cement	10,449.9	2	5224.9	55.1	<0.001
Cure method	51.1	1	51.1	0.538	0.465
Cement*cure method	33,925.2	2	16,962.6	178.7	<0.001

Table 3: *Two-Way Analysis of Variance: Simulated Generalized (Bulk) Wear, Facet Depth*

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F*-Ratio	*p*
Cement	1.97	2	0.986	49.8	<0.001
Cure method	0.024	1	0.024	1.195	0.277
Cement*cure method	6.78	2	3.39	171.0	<0.001

wear. The Proscan 2100 was used to make pretest and posttest scans, and the digitized surface contours were exported for examination with AnSur 3D software. Cement VL and FD were determined on the resin cement in the slit (gap) space as described above for the simulated generalized (bulk) wear model.

SEM

Specimens of each of the three self-adhesive resin cements were prepared for argon-ion etching and SEM examinations at Nihon University School of Dentistry (Tokyo, Japan). The three resin cements examined in this manner were not from the same lot numbers as the materials subjected to wear simulation studies and postwear SEM examinations at Creighton University School of Dentistry (Omaha, NE, USA). The lot numbers of the cement materials for the argon-ion etching SEM examinations were as follows: LA: 1402271, ME: 4394312, and RU: 497681.

The surfaces of the light-cure cements were polished to a high gloss with abrasive discs (Fuji Star Type DDC, Sankyo Rikagaku Co Ltd, Saitama, Japan) followed by a series of diamond pastes down to 0.25-μm particle size (DP-Paste, Struers, Ballerup, Denmark). The polished surfaces were then subjected to argon-ion beam etching (IIS-200ER, Elionix, Tokyo, Japan) for 45 seconds with the ion beam directed at the polished surfaces (accelerating voltage of 1.0 kV, ion current density of 0.4 mA/cm^2). The surfaces were then coated in a vacuum evaporator with a thin film of gold. Observations were made with a scanning electron microscope (FE-8000, Elionix) using an operating voltage of 10 kV and a magnification of 5000×.

SEM examinations were completed at Creighton University School of Dentistry on the wear facets of the three resin cement materials (light-cured and chemical-cured) following simulated generalized wear using the two wear models. After the wear analysis, representative samples of each material were sputter coated with gold and palladium using an Emitech SC7620 Mini Sputter Coater (Quorum Technologies, Ashford, UK). The coated wear specimens were then examined near the center of the wear facet with a TM3000 Tabletop Microscope (Hitachi-High Technologies Corp, Tokyo, Japan) using an accelerating voltage of 15 kV and magnifications of 2,500× and 5,000×.

Data Analysis of Simulated Wear

A two-way analysis of variance (ANOVA) (factors: [1] cement material and [2] cure method) and Tukey *post hoc* tests were used for data analysis of VL and FD of both light-cure and chemical-cure groups for both wear models. In addition, Pearson correlation tests were used to determine the relationship between the two wear models for both VL and FD of the light-cure and chemical-cure groups.

RESULTS

Generalized (Bulk) Wear Simulation

The two-way ANOVA of the generalized (bulk) wear data for both VL and FD showed a significant effect ($p<0.001$) for the factor of resin cement and the interaction of the resin cement and cure method. There was not a significant effect for the factor of cure method for either VL ($p=0.465$) or FD ($p=0.277$). The two-way ANOVA values are shown in Tables 2 and 3.

The results of the simulated generalized (bulk) wear are presented in Table 4. The VL (mm^3) and FD (μm) for the light-cure groups were as follows: VL: RU 0.631 (0.094), LA 0.692 (0.112), and ME 1.046 (0.141) and FD: RU 43.6 (6.5), LA 47.0 (7.7), and ME 72.5 (9.9). VL and FD for light-cure RU and LA were statistically similar ($p>0.05$). ME exhibited statistically ($p<0.05$) greater VL and mean FD than RU and LA for the light-cure cements.

The VL (mm^3) and FD (μm) for the chemical-cure groups were as follows: VL: LA 0.741 (0.105), RU 1.231 (0.234), and ME 1.305 (0.143) and FD: LA 50.7 (7.2), RU 84.5 (16.1), and ME 91.7 (10.2). For the chemical-cure cements, RU and ME show statisti-

Table 4: *Simulated Generalized (Bulk) Wear of Self-Adhesive Resin Cements (n=20)*[a]

Resin Cement	Light-Cure		Chemical-Cure	
	Volume Loss (mm^3)	Facet Depth (μm)	Volume Loss (mm^3)	Facet Depth (μm)
RU	0.631 (0.094) aA	43.6 (6.5) aA	1.231 (0.234) aB	84.5 (16.1) aB
LA	0.692 (0.112) aA	47.0 (7.7) aA	0.741 (0.105) bA	50.7 (7.2) bA
ME	1.046 (0.141) bA	72.5 (9.9) bA	1.305 (0.143) aB	91.7 (10.2) aB

[a] *Lowercase letters in vertical columns are not different at the 5% significance level. Same uppercase letters between columns indicate no difference (5% significance level) in light-cure vs chemical-cure of the same cement.*

cally ($p<0.05$) greater VL and FD than LA. Light curing of the RU and ME resin cements appeared to be more effective than chemical curing in limiting simulated generalized (bulk) wear. The FD and VL of RU were statistically greater ($p<0.05$) and nearly double for chemical-cure RU resin cement when compared to light curing. When comparing LA generalized wear of light-cure and chemical-cure cement, the results were statistically ($p>0.05$) similar for both VL and FD.

Slit (Gap) Model: Generalized Wear Simulation

The two-way ANOVA for both VL and FD using the slit (gap) model for generalized wear showed a significant effect for the factors of resin cement ($p<0.001$), cure method ($p=0.002$), and the interaction of resin cement and cure method ($p<0.001$). The two-way ANOVA values are shown in Tables 5 and 6.

The results of the slit (gap) model generalized wear are presented in Table 7. The VL (mm^3) and FD (μm) for the light-cure groups were as follows: VL: RU 0.030 (0.006), LA 0.031 (0.006), and ME 0.041 (0.009) and FD: RU 49.6 (5.7), LA 57.2 (8.4), and ME 70.9 (10.7). VL in the slit model for light-cure RU and LA was statistically similar ($p>0.05$). ME exhibited statistically ($p<0.05$) greater VL than RU and LA for the light-cure cements. RU exhibited the least slit (gap) model FD wear of the light-cure cements.

The slit model VL (mm^3) and FD (μm) for the chemical-cure groups were as follows: VL: LA 0.031 (0.004), ME 0.038 (0.007), and RU 0.045 (0.009) and FD: LA 53.9 (6.7), ME 63.5 (9.1), and RU 74.2 (12.9). The VL and mean FD of chemical-cure LA were significantly less ($p<0.05$) than RU and ME. RU exhibited the greatest VL and FD ($p<0.05$) when the chemical-cure groups were compared to light-cure groups while those of both LA and ME were essentially the same ($p>0.05$).

The results of the Pearson correlation tests are presented in Table 8. A strong relationship was found between the generalized (bulk) wear model and the slit (gap) model for the light-cure groups for VL ($r=0.999$) and FD ($r=0.968$). A good relationship was found between the two wear models for the chemical-cure groups for VL ($r=0.799$) and FD ($r=0.752$).

SEM Observations

The ultrastructure examinations with argon-ion etching SEM revealed morphological differences in filler components of the cements (Figure 6a–c). All three resin cements exhibited a wide variety of filler particle sizes and shapes. The particle size distribution of ME appeared to include larger particles than either LA or RU. The ultrastructure micrographs demonstrated that the filler components were different, and these compositional differences may have influenced the wear characteristics of these materials.

The SEM exanimations of the worn surfaces of all three of the resin cements showed evidence of particle loss (plucking) from the simulated gener-

Table 5: *Two-Way Analysis of Variance: Slit (Gap) Model Generalized Wear, Volume Loss*

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F*-Ratio	*p*
Cement	0.0015	2	0.000749	14.916	<0.001
Cure method	0.000529	1	0.000529	10.541	0.002
Cement*cure method	0.0016	2	0.000828	16.495	<0.001

Table 6: *Two-Way Analysis of Variance: Slit (Gap) Model Generalized Wear, Mean Facet Depth*

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F*-Ratio	*p*
Cement	3143.047	2	1571.524	19.745	<0.001
Cure method	813.49	1	813.490	10.221	0.002
Cement*cure method	5637.538	2	2818.769	35.416	<0.001

Table 7: *Slit (Gap) Model: Generalized Wear of Self-Adhesive Resin Cements (n=20)*[a]

Resin Cement	Light-Cure		Chemical-Cure	
	Volume Loss (mm^3)	Facet Depth (μm)	Volume Loss (mm^3)	Facet Depth (μm)
RU	0.030 (0.006) aA	49.6 (5.7) aA	0.045 (0.009) bB	74.2 (12.9) aB
LA	0.031 (0.006) aA	57.2 (8.4) bA	0.031 (0.004) aA	53.9 (6.7) bA
ME	0.041 (0.009) bA	70.9 (10.7) cA	0.038 (0.007) cA	63.5 (9.1) cA

[a] *Lowercase letters in vertical columns are not different at the 5% significance level. Same uppercase letters between columns indicate no difference (5% significance level) in light-cure vs chemical-cure of the same cement.*

alized wear with both wear models (Figures 7a–d, 8a–d, and 9a–d). There were also microcracks on the resin surface that most likely resulted from wear challenge fatigue stress. There was no apparent difference in worn surface morphology between the two wear models or when comparing light-cure and chemical-cure surfaces for each cement (Figures 7a–d, 8a–d, and 9a–d). Any observed differences between the light-cure and chemical-cure surfaces, as well as comparisons between the bulk and slit model worn surfaces, were subtle. There did appear to be a qualitative morphological difference for RU, where the chemical-cure surfaces seemed to have more filler particle plucking than light-cure surfaces. This observation is consistent with the differences in wear values for RU between light-cure and chemical-cure specimens (Tables 9 and 10).

DISCUSSION

Two methods of wear simulation were used in this laboratory study to assess the relative wear resistance of three self-adhesive resin cements. Wear generated using a new slit (gap) model was compared to a commonly used simulated generalized (bulk) wear method. In both models, a flat-ended stainless-steel antagonist was used to produce wear using a water slurry of PMMA beads as the abrasive media. Both light-cure and chemical-cure specimens of the three self-adhesive resin cements were assessed using SEM examinations and wear analysis. The rank order (RU-LA-ME) of wear (VL and FD) for the three resin cements was the same for the two test models when the cements were light-cured (Tables 4 and 7). The rank order of wear using the chemical-cure method was not the same as the light-cure groups when the results of the two wear methods were examined (Tables 4 and 7).

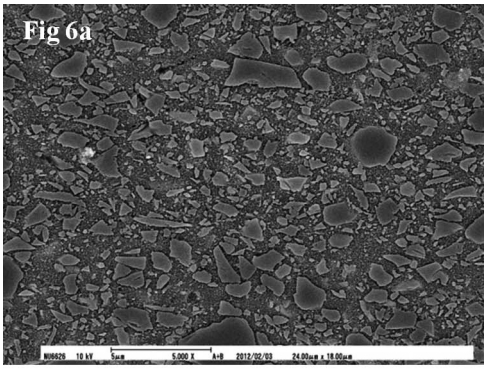
Table 8: *Pearson's Correlations (r): Generalized (Bulk) and Slit (Gap) Wear Models*

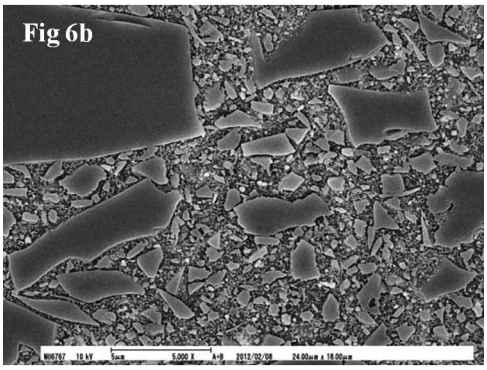
Polymerization Method	Volume Loss	Facet Depth
Light-cure	0.999	0.968
Chemical-cure	0.799	0.752

The wear data of the resin cements for both the simulated (bulk) wear model and the slit (gap) model showed that the LA cement, when light-cured and chemical-cured, were similar and not statistically different ($p>0.05$) for FD and VL (Tables 4, 7, 9, and 10). The generalized (bulk) wear values of RU nearly doubled with the chemical-cure method when compared to light curing alone (Tables 4 and 9). The results of the slit (gap) model wear (VL and FD) of RU showed a 50% increase for the chemical-cure group when compared to light curing (Table 10). ME exhibited the highest wear values of the three materials in this study with both wear models (Tables 4 and 7), showing about a 25% increase in both VL and FD with the generalized (bulk) wear method (Table 9). A decrease in wear (VL and FD) for ME was found with the slit (gap) model for chemical curing when compared to light curing (Table 10).

Previous studies utilizing the generalized (bulk) wear model have used 400,000 cycles for testing of resin-based materials.[18-23] Leinfelder and Suzuki[22] have reported that for resin composite materials, there was a high level of agreement between wear generated with 400,000 cycles in the Alabama simulator and 3 years of clinical service. Over the years, most of the laboratory testing with the Alabama machine has been done using 400,000 cycles.

With the new slit (gap) model utilized in this study, 100,000 cycles were used for testing versus the 400,000 cycles. There was a twofold reason for reducing the number of cycles with the slit (gap) test model in the preliminary or initial testing of the new wear model: 1) force concentration from the antagonist was applied to a much smaller area of resin cement than the traditional generalized (bulk) model, and 2) testing was expedited when compared to using 400,000 cycles. The





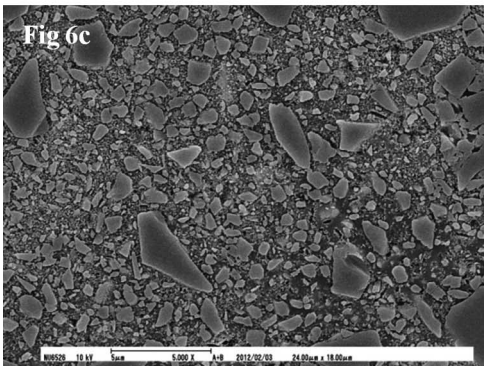


Figure 6a. *G-CEM Link Ace: argon-ion etched surface (5000×).*

Figure 6b. *Maxcem Elite: argon-ion etched surface (5000×).*

Figure 6c. *Rely X Unicem 2 Automix: argon-ion etched surface (5000×).*

results clearly showed that the slit (gap) model could discriminate wear among the three self-adhesive resin cements using 100,000 cycles (Table 7).

Previous studies[24,25] comparing degree of conversion for light curing and chemical curing have generally found that light curing produces a significantly higher degree of conversion than chemical curing alone for dual-cure cements. Ferracane and others[13,26] have also reported that wear resistance and mechanical properties of resin composites are increased by improving the degree of conversion. The ability of dual curing cements to effectively cure in the chemical set mode for indirect restorations is vitally important to the long-term success of restorative procedures where light curing is not possible or is limited.

A critical factor in the setting reaction for chemical curing of self-adhesive resin cements is the influence of the amine initiator. It is speculated from earlier studies showing a superior degree of conversion of dual-cured cements with photoactivation compared to chemical curing that this difference was caused by acidic monomers impacting negatively on the chemical-cure setting reaction by lowering the pH.[15,24,25] In examining simulated generalized (bulk) wear in the present study, two of the three self-etch adhesive systems (RU and ME) showed significantly ($p<0.05$) less wear (VL and FD) for light-cure groups when compared to chemical-cure groups (Table 4). The simulated (bulk) wear of LA was slightly greater in the chemical-cure group when compared to the light-cure material (Table 4), but this difference was not significant ($p>0.05$). The wear of LA in the slit (gap) model was essentially the same for the light-cure and chemical-cure groups. The wear of RU in the slit (gap) model (Table 7) exhibited the same pattern as the generalized (bulk) model (Table 4) with the chemical-cure cement showing significantly ($p<0.05$) more wear than the light-cure cement. The wear of ME was slightly less ($p>0.05$) in the chemical-cure group in the slit (gap) model (Table 7) when compared to the light-cure counterpart. It is interesting to note that the wear of chemically-cured LA in both the generalized (bulk) wear model and the slit (gap) wear model was significantly less than both RU and ME (Tables 4 and 7). This would suggest that the

Table 9: *Generalized (Bulk) Wear Model: Percent Change, Light-Cure to Chemical-Cure (n=20)*

Resin Cement	Volume Loss (mm^3)			Facet Depth (μm)		
	Light-Cure	Chemical-Cure	% Change	Light-Cure	Chemical-Cure	% Change
RU	0.631 (0.094)	1.231 (0.234)	95.1	43.6 (6.5)	84.5 (16.1)	93.8
LA	0.692 (0.112)	0.741 (0.105)	7.1	47.0 (7.7)	50.7 (7.2)	7.9
ME	1.046 (0.141)	1.305 (0.143)	24.8	72.5 (9.9)	91.7 (10.2)	26.5

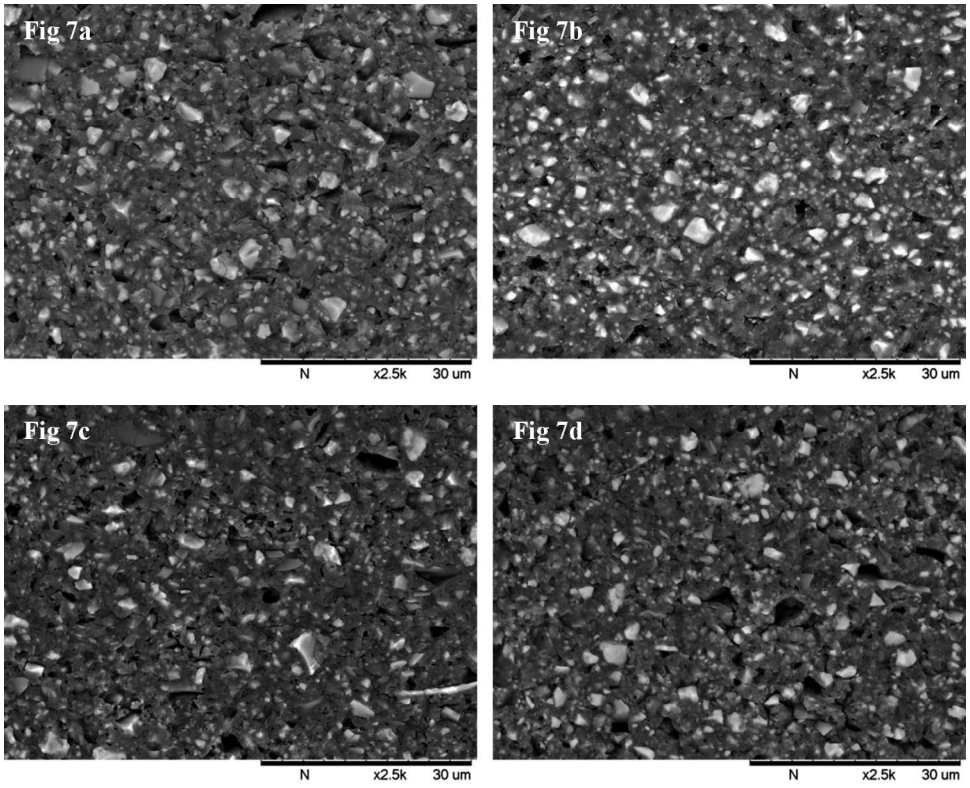


Figure 7a. *G-CEM Link Ace/light-cure: generalized (bulk) wear (400,000 cycles) near center of wear facet (2500×).*

Figure 7b. *G-CEM Link Ace/chemical-cure: generalized (bulk) wear (400,000 cycles) near center of wear facet (2500×).*

Figure 7c. *G-CEM Link Ace/light-cure: slit (gap) generalized wear (100,000 cycles) near center of wear facet (2500×).*

Figure 7d. *G-CEM Link Ace/chemical-cure: slit (gap) generalized wear (100,000 cycles) near center of wear facet (2500×).*

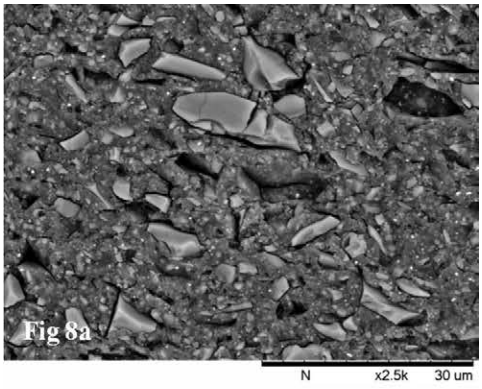
polymerization reaction with chemical curing for LA is more effective than RU and ME. Thus, the null hypotheses—1) there will not be a significant difference in wear values among three dual-cured self-adhesive resin cements and 2) wear of self-adhesive resin cements using light-cure or chemical-cure methods will not be different—are rejected for RU and ME in the generalized (bulk) wear model but not for LA and rejected for RU in the slit (gap) wear model.

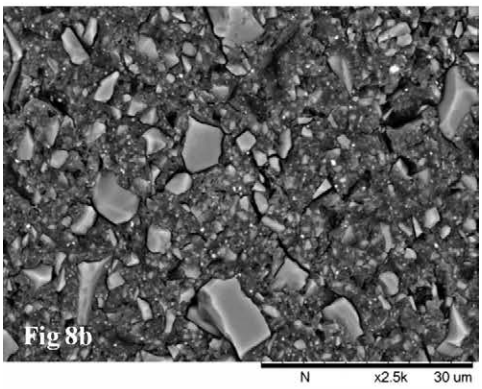
Belli and others[14] reported data for self-adhesive resin cements using a gap model for both toothbrush abrasion and the ACTA wear method. They related that self-adhesive resin cements exhibited good wear resistance to toothbrush abrasion but showed much more wear under the heavier loading with the ACTA test. They also reported that no correlation (R^2=0.0567) was found between the two test methods. In the present study comparing the generalized (bulk) wear model and the slit (gap) model, a strong correlation (Pearson) was found between the light-cure groups for both VL (r=0.999) and FD (r=0.968). For the chemical-cure groups, the correlations between the two models were r=0.799 for VL and r=0.752 for FD. While the associations for the chemical-cure groups were not as robust as for the light-cure groups, the relationships were good for the chemical-cure groups with the two wear methods used in this study.

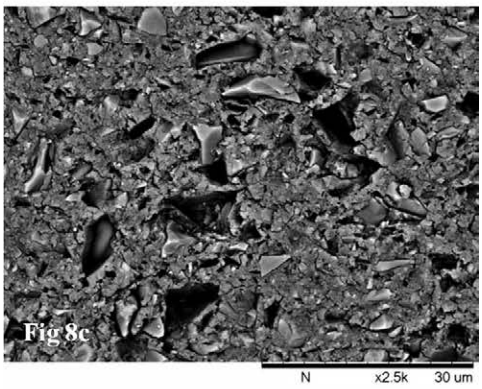
Barkmeier and others[18-20] have conducted several studies with the generalized (bulk) wear model used in this study to evaluate the wear resistance of resin composite materials. The new slit or gap model was used in the present study to more closely parallel a clinical situation in the assessment of wear resistance of self-adhesive resin cements. The new slit (gap) model delivers the wear challenges with the

Table 10: *Slit (Gap) Wear Model: Percent Change, Light-Cure to Chemical-Cure (n=20)*

Resin Cement	Volume Loss (mm^3)			Facet Depth (μm)		
	Light-Cure	Chemical-Cure	% Change	Light-Cure	Chemical-Cure	% Change
RU	0.030 (0.006)	0.045 (0.009)	50.0	49.6 (5.7)	74.2 (12.9)	49.6
LA	0.031 (0.006)	0.031 (0.004)	0.0	57.2 (8.4)	53.9 (6.7)	−5.8
ME	0.041 (0.009)	0.038 (0.007)	−7.3	70.9 (10.7)	63.5 (9.1)	−10.4







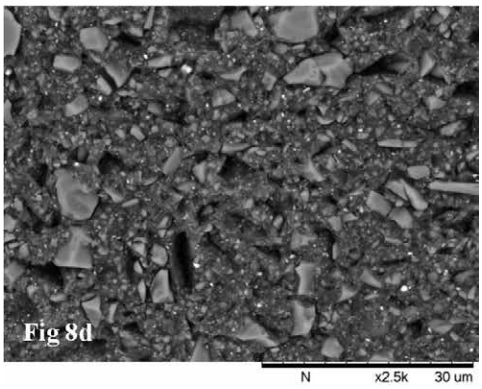


Figure 8a. *Maxcem Elite/light-cure: generalized (bulk) wear (400,000 cycles) near center of wear facet (2500×).*

Figure 8b. *Maxcem Elite/chemical-cure: generalized (bulk) wear (400,000 cycles) near center of wear facet (2500×).*

same stainless-steel antagonist tip used in the generalized (bulk) wear simulation model. The primary difference is the area of cement exposed to the wear process. Overall, the correlation of VL and FD using the two methods was excellent. While the goal of using the slit (gap) model was to more closely replicate the type of abrasion that may occur in the oral cavity, the results indicate that the wear resistance of resin cements can be assessed with the standard generalized (bulk) wear model. The slit (gap) method does not appear to offer any real advantages in the assessment of the wear resistance of self-adhesive resin cements. However, the slit (gap) model reaffirms that abrasive wear of a thin film of a resin cement in marginal closure areas remains an issue for long-term clinical performance of cemented restorations.

Wear resistance of resin cements may be influenced by water sorption of these materials. Ferracane and others[1] have cautioned that the hydrophilic nature of resin cements, due to the low pH of cured material, can result in excessive water sorption, which may cause material swelling and compromised mechanical properties. These authors indicated that the concentration of acidic monomers must be balanced to effectively etch mineralized tooth structures for bonding but also avoid hydrophilicity in the cured cement. Studies have shown that some resin cements are more prone to water sorption and subsequent degradation than others.[16,27-31] Zorzin and others[16] have related that resin cement specimens that are desiccated during the acid-base reaction phase could result in extraction of water produced during the setting reaction and that this could interfere with setting and pH neutralization kinetics. In the present laboratory study, all the specimens were fabricated on the bench and stored at room temperature for 24 hours before polishing and testing. The materials were rehydrated during the wear-testing procedure. However, in future studies, the storage conditions and resultant effects on these types of specimens, especially the chemical-cure groups, should be investigated. The wear mechanics, as related to hydration during the setting reaction and subsequent water sorption, needs further attention. Another potential influence in wear characteristics using the slit (gap) model

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Figure 8c. *Maxcem Elite/light-cure: slit (gap) generalized wear (100,000 cycles) near center of wear facet (2500×).*

Figure 8d. *Maxcem Elite/chemical-cure: slit (gap) generalized wear (100,000 cycles) near center of wear facet (2500×).*

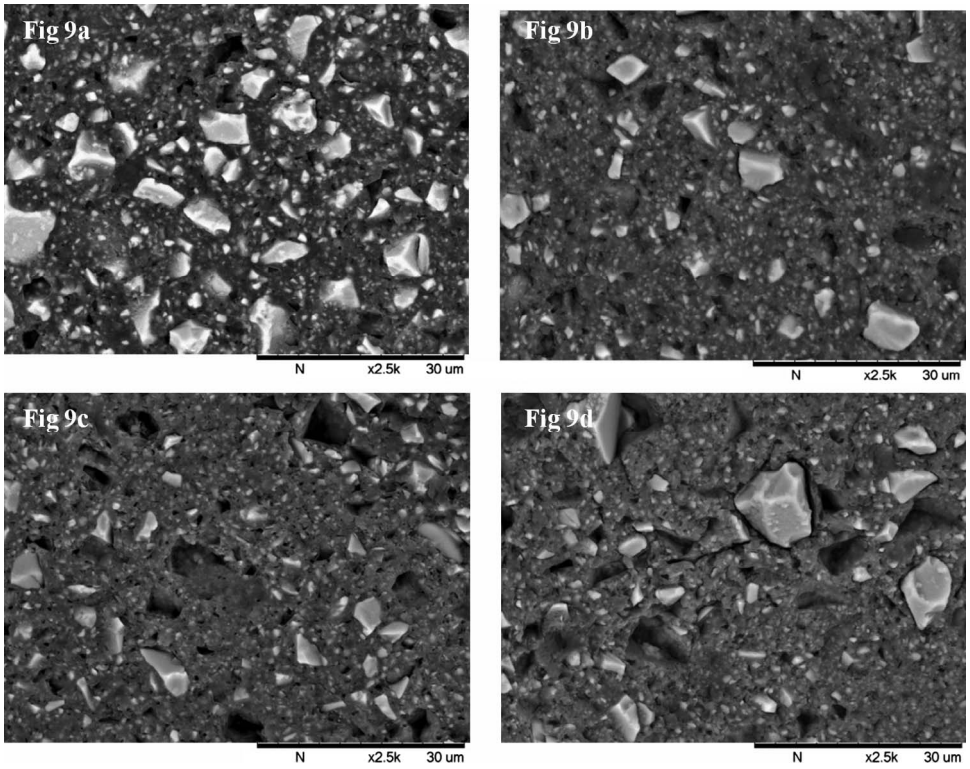

Figure 9a. *RelyX Unicem2 Automix/ light-cure: generalized (bulk) wear (400,000 cycles) near center of wear facet (2500×).*

Figure 9b. *RelyX Unicem2 Automix/ chemical-cure: generalized (bulk) wear (400,000 cycles) near center of wear facet (2500×).*

Figure 9c. *RelyX Unicem2 Automix/ light-cure: slit (gap) generalized wear (100,000 cycles) near center of wear facet (2500×).*

Figure 9d. *RelyX Unicem2 Automix/ chemical-cure: slit (gap) generalized wear (100,000 cycles) near center of wear facet (2500×).*

would be the effect of promoting adhesion to the internal surfaces of the chamber in the slit (gap) specimen holder. Eliminating the separating medium and including a procedure to promote bonding between the cements and the stainless-steel fixtures may improve adhesion and more effectively mimic clinical situations. This procedural change may impact of the simulated wear values in the slit (gap) model and should be investigated further in future studies.

CONCLUSIONS

A generalized (bulk) wear simulation model and a new slit (gap) wear model showed differences ($p<0.05$) in the relative wear resistance of three self-adhesive resin cements and between light cure and chemical-cure techniques. Both wear models provided valuable information regarding the wear resistance of self-adhesive resin cements.

Acknowledgement

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Regulatory Statement

This study was conducted at the Creighton University School of Dentistry and Nihon University School of Dentistry.

Conflict of Interest

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

(Accepted 22 February 2015)

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Departments

ERRATUM

Operative Dentistry would like to clarify author order in "Brambilla E, Ionescu A, Cazzaniga G, & Ottobelli M (2016) Influence of light-curing parameters on biofilm development and flexural strength of a silorane-based composite *Operative Dentistry* **41(2)** 219-227. The correct author order should be "Ottobelli M, Ionescu A, Cazzaniga G, & Brambilla E." *Operative Dentistry* apologizes for any confusion.

LETTER TO THE EDITOR

Dear Dr Platt:

I read with interest the following manuscript: Torres CR, & Borges AB (2015) Color masking of developmental enamel defects: A case series *Operative Dentistry* **40(1)** 25-33 (my special thanks to the authors for such a great clinical manuscript).

I have to disagree with the credit given to references 13 and 14 on page 26. The resin infiltration concept is at least 40 years old. Robinson and others[1] in 1976 etched enamel with hydrochloric acid (HCl) and infiltrated this etched enamel with a resorcinol-formaldehyde resin as a potential cariostatic treatment. Then, in 1987, Croll[2] used a clear resin sealant on etched enamel to saturate the surfaces with resin. Among several research papers on the topic of microabrasion followed by resin infiltration published in the 2000s, I would highlight two from the same research group. In 2007, Paris and others[3] used confocal microscopy to (elegantly) study resin infiltration of carious lesions using 37% H_3PO_4 or 15% HCl to etch enamel, followed by immersion in ethanol for 30 seconds and the application of the commercial dentin adhesive ExciTE (Ivoclar Vivadent). In 2009, Paris and Meyer-Lueckel[4] described the masking of white spots with resin infiltration using 15% HCl etching followed by a drying step with ethanol and a very-low-viscosity light-cured resin (tetraethylene glycol dimethacrylate).

I hope that this clarification gives the deserved credit to the authors of the missing references, especially to the papers of Robinson and others[1] and Croll[2], which are often overlooked.

Sincerely,

Jorge Perdigão

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Author Response

Dear Dr Jorge Perdigão,

Thank you for expressing your opinion. Our intention in citing references 13 and 14 in the original manuscript was to reference the process of penetration, hardening, and inhibiting lesion progression, as also demonstrated in these studies. Nevertheless, the author of the letter to the editor is completely correct about the development of the infiltration concept. We apologize for this mistake, because we used the term "introduced" in the sentence, and we agree that the credit should be given to the authors referenced in the letter to the editor.

Best regards,

Dr Borges and coauthors

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Evaluation of Bond Strength and Microleakage of a Novel Metal-titanate Antibacterial Agent

S Deng • KH Chung • DCN Chan • C Spiekerman

Clinical Relevance: The novel antibacterial nanoparticulate metal-titanate complexes under investigation will allow clinicians to tackle the composite longevity problem at its weakest interface.

doi: http://dx.doi.org/10.2341/14-257-L

Effect of Cigarette Smoke on Resin Composite Bond Strength to Enamel and Dentin Using Different Adhesive Systems

JD Theobaldo • A Catelan • U Rodrigues-Filho • GM Marchi • DANL Lima • FHB Aguiar

Clinical Relevance: The exposure of dentin to cigarette smoke influences adhesive bonding strength. However, cigarette smoke does not influence the bond strength to enamel.

doi: http://dx.doi.org/10.2341/15-056-L

Longevity of Bonding of Self-adhesive Resin Cement to Dentin

TC Simões • ÍV Luque-Martinez • RR Moraes • ATG Sá • AD Loguercio • SK Moura

Clinical Relevance: Regardless of the cementing strategy, the durability of bonding to root canal dentin may be influenced by the dentin treatment protocol.

doi: http://dx.doi.org/10.2341/14-266-LR

Composite Replacement of Amalgam Restoration Versus Freshly Cut Dentin: An In Vitro Microleakage Comparison

H Redwan • DN Bardwell • A Ali • M Finkelman • S Khayat • H-P Weber

Clinical Relevance: Bonding to dentin under replaced amalgam restorations may be as effective as bonding to freshly cut dentin.

doi: http://dx.doi.org/10.2341/14-278-L

Mechanical Properties and Sliding-impact Wear Resistance of Self-adhesive Resin Cements

T Furuichi • T Takamizawa • A Tsujimoto • M Miyazaki • WW Barkmeier • MA Latta

Clinical Relevance: Mechanical properties and wear resistance are important parameters for the selection of resin luting cements. For wear resistance, the self-adhesive resin cements, apart from GL, showed significantly lower values than the conventional resin cements.

doi: http://dx.doi.org/10.2341/15-033-L

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Evaluation of Bond Strength and Microleakage of a Novel Metal-titanate Antibacterial Agent

S Deng • KH Chung
DCN Chan • C Spiekerman

Clinical Relevance

The novel antibacterial nanoparticulate metal-titanate complexes under investigation will allow clinicians to tackle the composite longevity problem at its weakest interface.

SUMMARY

Objectives: To evaluate the effect on both bond strength and microleakage of incorporation of a novel antibacterial nanoparticulate metal-titanate complex (nMT) into a dental adhesive system.

Materials and Methods: Eighty extracted human molars were prepared to determine whether incorporation of nMT into bonding agents can affect shear bond strength (SBS) and adhesive strength fatigue. SBS was measured with a universal testing machine, and the peak force at failure was recorded. An electromechanical fatigue machine was used for cyclic loading treatment of specimens. Differences in the SBS values among groups were identified using analysis of variance and Tukey post hoc analyses (α=0.05). Twenty standard Class V cavities were restored to examine microleakage when the primer/bonding resin was modified with 10 wt% nMT. Microleakage at the enamel and dentin margins was calculated as a percentage of the full length of the cavity. Results of the microleakage experiment were analyzed with paired and independent sample *t*-tests (α=0.05).

Results: The mean (± standard deviation) shear bond strength values of before fatigue and after fatigue ranged from 21.9 (2.5) MPa to 23.9 (3.8) MPa and from 17.1 (2.5) MPa to 17.7 (2.5) MPa respectively. No statistically significant differences in failure force were observed among groups (p=0.70). Microleakage under all conditions was significantly greater in the dentin margins than in the enamel margins (p<0.05). There was no evidence that microleakage differed between the experimental

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groups with modified primer and bonding resin.

Conclusions: Incorporating nMT into a dental adhesive system will not compromise the resin composite's tooth bonding and sealing ability.

INTRODUCTION

Biofilms are medically important because microbes in the biofilm state are more pathogenic than when they are planktonic (nonadherent and free floating). The National Institutes of Health reported[1] that bacteria growing as a biofilm cause 80% of infections in the body. Biofilms at the margin of an existing dental restoration give rise to secondary caries, which are the main complication necessitating replacement of composite fillings.[2,3] Currently there is a lack of research data both on 1) the effect of the cariogenic biofilm community structure and its metabolic processes on the tooth/resin composite interface and 2) the effect of the tooth/resin composite on biofilm formation and population distribution.

Monosodium titanate (MST) is an inorganic, particulate compound of titanium oxide ($NaTi_2O_5H$) with an amorphous core and crystalline surface. MST has a spherical morphology with an approximate diameter of 1-10 μm. Our team has developed microparticulate metal-titanate complexes as a new class of antibacterial agents.[4-7] The microparticulate gold (III)–loaded titanate complexes inhibit growth of oral bacteria at micromolar concentrations. We predict that nanoparticulate metal-titanate complexes (nMTs) in turn will be even more effective at inhibiting oral bacteria growth as such complexes have a significantly greater surface-to-volume ratio, resulting in more effective ion-exchange characteristics. This approach is innovative as nMTs are ceramic in nature and act like resin filler. In addition, because these complexes are not organic, degradation is not an issue, and thus they can have long-term effectiveness and also may be less likely than organic antibacterial agents to contribute to bacterial resistance.[8]

Dental composite restorations are becoming the material of choice for posterior restorations; thus, their longevity is important to patients, dentists, federal health agencies, and insurance companies. A survey of 24 prospective studies on the clinical performance of posterior resin composites published between 1996 and 2002 indicated that the primary reasons for composite failure were secondary caries, restoration fracture, and marginal defects.[2] Secondary caries was the principal cause of composite failure necessitating replacement of fillings; these caries are lesions at the margin of an existing restoration and usually occur in areas of biofilm stagnation.[9] For this reason, the cervical margins of restorations are commonly affected. In the oral cavity, mixed microbial biofilms can accumulate on hard and soft tissues and are involved in the pathogenesis of caries and periodontitis. A biofilm is an accumulation of bacteria, fungi, or protozoa on solid surfaces. In dentistry, two popular approaches to preventing biofilm formation are 1) to design a biomaterial that slowly releases an agent that is lethal to the approaching bacterial cells and 2) to develop a nonadhesive surface by modifying the surface chemistry of restorative materials.[10] Various chemical agents can affect bacterial adhesion indirectly by disrupting bacterial cell metabolism. Numerous materials have been impregnated with various antibiotics only to have most of the agent released over a very short time, thus providing no long-term effect.[11] Recent studies have shown that sublethal doses of antibiotics can induce bacterial resistance and actually enhance biofilm formation. The potential negative consequences of bacterial resistance to antibiotics are dire because they put all of society at risk.[12]

Metal-based antibacterials are an attractive alternative. Metal ions have chemical properties that inhibit bacterial growth.[13] The unique binding, coordination, and redox properties make development of bacterial resistance less likely and predict effectiveness across a broad bacterial spectrum. For example, Ag(I) and Hg have a long history as antibacterials.[14] However, fears of systemic toxicity have limited Ag(I) and Hg use in recent years.[15] Other metal ions, such as Au(III), Pd(II), and Pt(IV), have binding properties, coordination chemistries, and redox properties that suggest they also would be effective antibacterials. Unfortunately, development of new metal-based antibacterials has been severely impeded because of previous controversies and fears. Yet, given the increasing resistance of bacteria to organic antibacterials, metal-based antibacterials are a promising alternative. If systemic toxicity could be limited and therapeutic indices were optimized, metal ions and their associated compounds could emerge as a new powerful class of antibacterial agents.

The goal of this investigation was to evaluate the bond strength and microleakage of a kind of novel

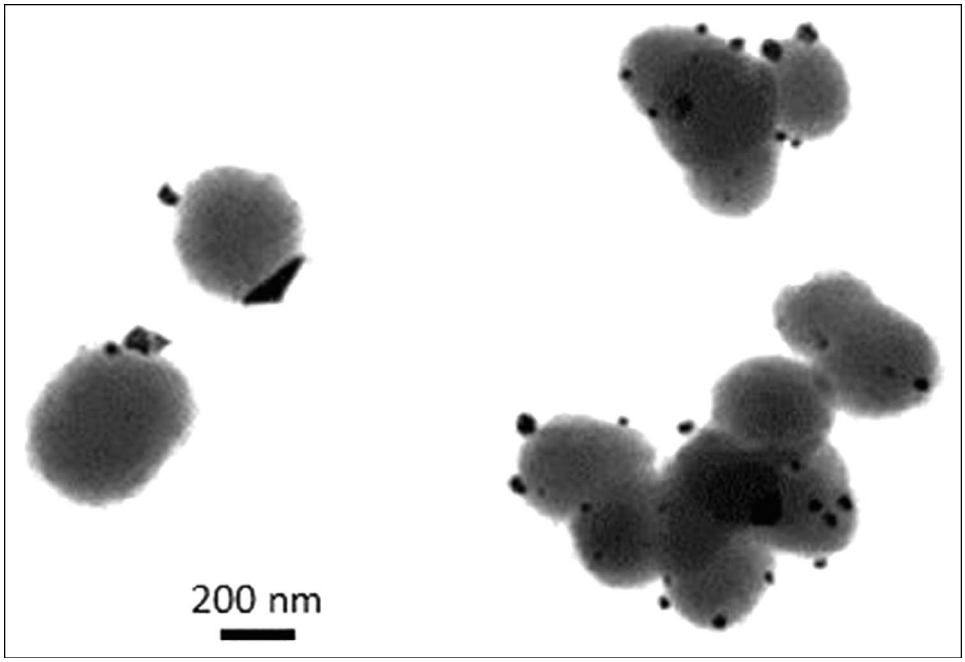


Figure 1. *Transmision electron microscopy (TEM) image of nanoparticulate gold-loaded monosodium titanate complexes.*

antibacterial nMT. This kind of nMT has been shown previously to have antimicrobial activity.[6]

METHODS AND MATERIALS

Experimental Materials

MSTs are particulate, micron-sized ion exchangers that may be useful solid-phase platforms for delivery of metal ions to inhibit bacterial growth.[4-7] The synthesis of micro- and nano-titanate particles based on a unique low-temperature (<80°C) sol-gel process has been reported.[16] The process produces titanates with superior ion-exchange characteristics compared to those produced using hydrothermal materials. Previous work[6] has shown that nanoparticulate titanate complexes with loaded gold and cisplatin have antimicrobial activities. An example of nanoparticulate gold-loaded MST complexes (nMTs) is illustrated in Figure 1. A 10% wt/wt nMT powder was added to Primer (All-Bond 2®, Bisco Inc, Schaumburg, IL, USA) and bonding resin (D/E resin, Bisco) and mixed in a dark room.

Shear Bond Strength (SBS) Test

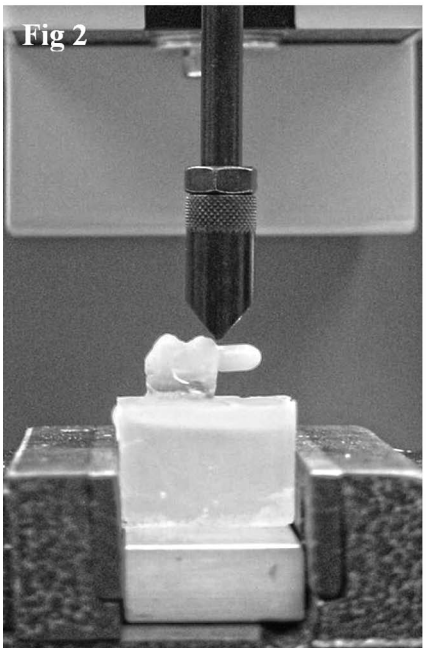
The SBS test was conducted in three phases as the metal-titanate complexes were processed and available. In the first phase, we examined whether incorporation of MST affects the bond strength. In the second phase, we compared the effects of adding nMT. In the third phase, we examined the effect of cyclic loading on bond strength.

In the first and second phases, 80 extracted human molars were prepared with a coarse diamond bur at axial surfaces to create an approximately 8-mm-diameter enamel or dentin flat surface that was parallel with the long axis of each tooth. The surfaces were further wet polished up to 600 grit. Two surfaces from each tooth were selected for the bonding test, and the surfaces were etched with 32% H_3PO_4 (Uni-Etch, Bisco) for 15 seconds. They were thoroughly rinsed and the excess water removed with a brief burst of air. The modification of an adhesive system and bonding treatment are described in Table 1. Filtek Supreme Plus (3M ESPE, St Paul, MN, USA) was injected into a

Table 1: *List of Experimental Groups for Shear Bond Strength Test in This Study*

Groups	Bonding Treatment
Enamel (control)	Etching, priming, and bonding[a]
Enamel + 1	Etching, priming, and bonding with (D/E resin+10 wt% MST)
Enamel + 2	Etching, priming, and bonding with (D/E resin+10 wt% nMT)
Dentin (control)	Etching, priming, and bonding[a]
Dentin + 1	Etching, priming with (primer+10 wt% MST), and bonding
Dentin + 2	Etching, priming with (primer+10 wt% nMT), and bonding

Abbreviations: MST, monosodium titanate; nMT, nanoparticulate gold-loaded monosodium titanate complex.
[a] According to the manufacturer's instruction for the Universal Dental Adhesive System: All-Bond 2®.



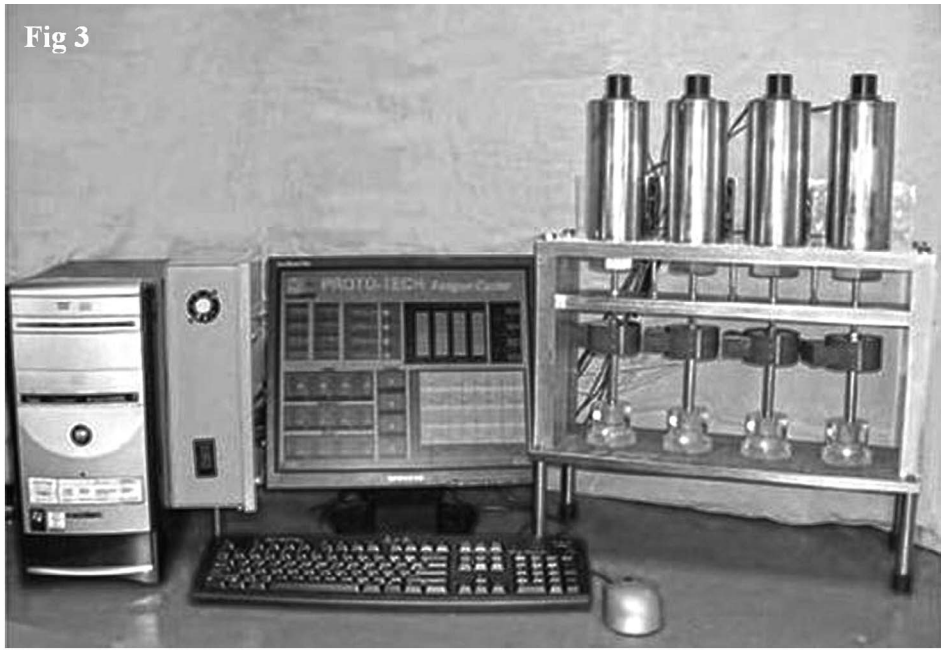


Figure 2. *Set up for shear bond strength testing.*

Figure 3. *A four-station fatigue cycler (Proto-tech, Portland, OR, USA) with DASYLab software (DASYLab, Norton, PA, USA).*

cellular gel capsule (4.4 mm in internal diameter, 12 mm in length) and attached perpendicularly on the etched enamel or dentin surface. The capsule was light-cured for 30 seconds on each surface (for a total of 120 seconds) using a LED light-curing unit (Elipar Frelight II, Dentsply, Konstanz, Germany) and stored in a water bath at 37°C for 24 hours. SBS was then measured with a universal testing machine (n=16, cross-head speed=5 mm/min), and the bond strength at failure was recorded (Figure 2).

In the third phase, a four-station electromechanical fatigue machine (Fatigue Cycler, Proto-tech, Portland, OR, USA) was used for cyclic loading treatment of specimens before the SBS testing. Specimens were mounted into a custom fixture inside a 37°C water chamber. The load for each station was adjusted with computerized software (DASYLab, Norton, PA, USA). The lower load limit was set at zero, and the maximum load applied was 70 N (which was approximately 30% of the average of the SBS values determined from the first and second phases, above). The load was applied at a rate of 1.2 Hz using a sine wave for 40,000 cycles (Figure 3). After cyclic loading, all specimens were stored in distilled water at 37°C for 24 hours and then the SBSs of postcyclic loading specimens were tested as described above. Differences in the SBS values among groups were identified using analysis of variance and Tukey post hoc analyses ($\alpha \leq 0.05$).

Microleakage Examination

Forty standard Class V cavities (4 × 2 × 2.5 mm) were prepared at the cemento-enamel junction (CEJ) on the buccal and lingual surfaces of 20 freshly extracted human molars. The cavities were prepared using diamond burs in a high-speed handpiece with water coolant. A single operator (SLD) performed all the cavity preparations and restorations. The specimens were randomly assigned to two study groups: group 1, primer (All-Bond 2®, Bisco) modified with 10 wt% nMT and group 2, bonding resin (D/E resin, Bisco) modified with 10 wt% nMT. Lingual cavities were treated by unaltered primer/adhesive resin as controls.

Cavities were then restored with resin composite (Filtek Supreme Plus) in the traditional incremental placement manner. The restorations were polished with aluminum oxide discs (Sof-Lex, 3M ESPE) and then stored in a water bath at 37°C for 24 hours to ensure adequate polymerization. The specimens were subjected to 500 cycles of thermocycling treatment between 5°C and 55°C with 30-second dwell time and a three-second transfer. Two coats of nail polish were then applied to the entire tooth surface within 1.0 mm of the restoration margins.

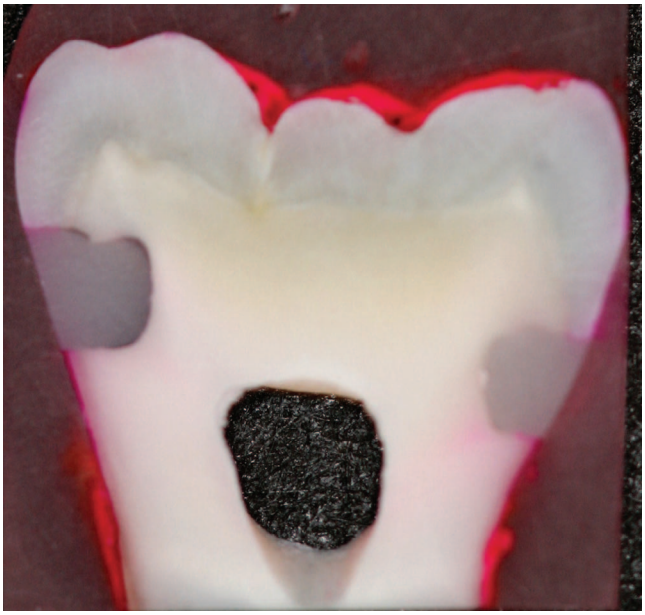


Figure 4. *Restored Class V cavities at the CEJ on the buccal and lingual surfaces to be evaluated for microleakage.*

The specimens were incubated separately in individual screw-capped tubes in a water bath at 37°C for 24 hours before immersion in a 0.2% Rhodamine B solution (Invitrogen, Carlsbad, CA, USA) for 24 hours. The specimens were then rinsed with distilled water for 10 minutes, dried, and embedded in a cold curing epoxy resin for two hours at 60°C. Blocks of resin were left at room temperature for six hours to achieve complete polymerization. Finally, two buccolingual sections were made through each restoration with a low-speed diamond saw (IsoMet, Buehler Ltd, Lake Bluff, IL, USA). Each tooth was sectioned into three pieces, producing four cross-sectional faces for evaluation. Sections were assessed for dye penetration with an optical microscope (Nikon Eclips E600, Tokyo, Japan) at ×20 magnification at the occlusal and cervical margins. A total of 160 scores for dentin margins and enamel margins were recorded. Microleakage at the enamel and dentin margins was calculated as a percentage of the full length of the cavity (Figure 4). The results of the microleakage examination were analyzed with paired and independent sample *t*-tests (α=0.05).

Operator Reliability Evaluation

To ensure consistency and reliability of the observations, 20 sections were chosen randomly to assess interoperator reliability. A second evaluator read each section under the same conditions. Correlation coefficients for both enamel and dentin percentages between the two evaluators (DCNC and SLD) were as follows: enamel%, $r = 0.915$; dentin%, $r = 0.936$. No statistically significant differences were observed between the two operators' evaluations (paired *t*-test, $p>0.05$). Following the interoperator reliability assessment, selected sections were also evaluated by a confocal laser scanning electron microscope (Zeiss510, Carl Zeiss Ltd, Thornwood, NY, USA) (Figure 5).

RESULTS

The mean (± standard deviation) SBS values of before fatigue and after fatigue ranged from 21.9 (2.5) MPa to 23.9 (3.8) MPa and from 17.1 (2.5) MPa to 17.7 (2.5) MPa, respectively. No statistically significant differences in failure force were observed among groups (p=0.70) (Figure 6). The results indicated that adding 10 wt% nMT to the adhesive system (either primer or bonding resin) did not affect the bond strength of the resin composite to either enamel or dentin.

The results of the microleakage experiment were analyzed with paired and independent sample *t*-tests (α=0.05). No significant differences were observed between test groups and control restorations (Table 2). No significant differences were observed between groups 1 and 2 in either dentin or enamel margins. However, strong evidence of differential leakage susceptibility was observed between enamel and dentin margins ($\alpha<0.01$). Microleakage under all conditions was significantly greater in the dentin margins than in the enamel margins ($p<0.05$). We found no evidence that microleakage differed between the experimental groups with modified primer and bonding resin.

DISCUSSION

Antimicrobial antibiotics, nanoparticles (NPs), polymers, and peptides have been developed to prevent and/or reduce bacterial growth and adhesion.[17-20] Metal-based antibacterials are a desirable choice because bacterial resistance to conventional antibiotics is a common problem. As metal complexes are inorganic, they can have long-term effectiveness as well as potentially reduced contribution to bacterial resistance.[8] Many recent studies have revealed that nanoparticles, especially silver NP (AgNP), have various properties that can be exploited for numerous biomedical applications. In one study,[21] the antibacterial activity of AgNP-modified hydrogel coatings was tested evaluating *in vitro* inhibition growth of *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*. The composite with AgNP was strongly antibacterial and greatly

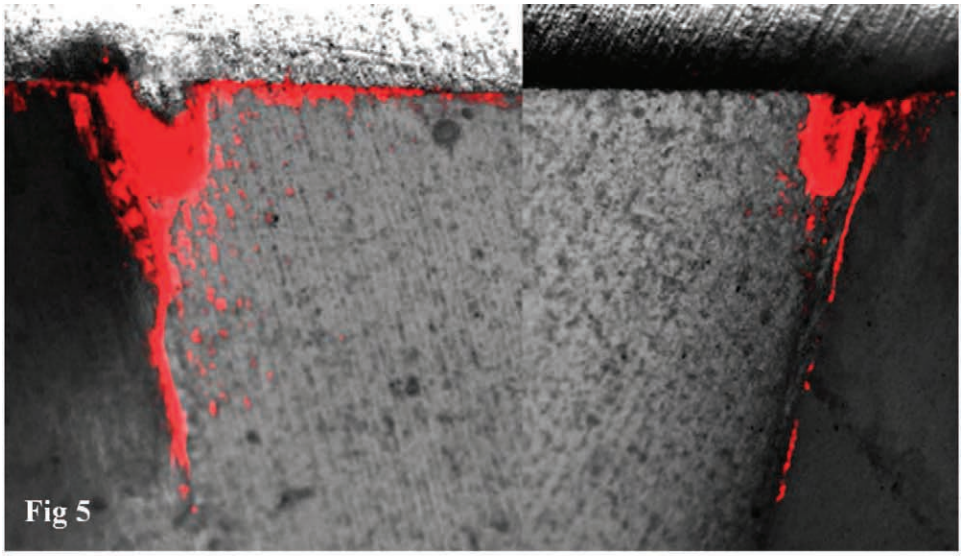


Figure 5. *Penetration of the 0.2% Rhodamine dye as indicated by confocal microscopy in the control enamel group compared to the treated enamel group. Note that the degree of penetration is very similar.*

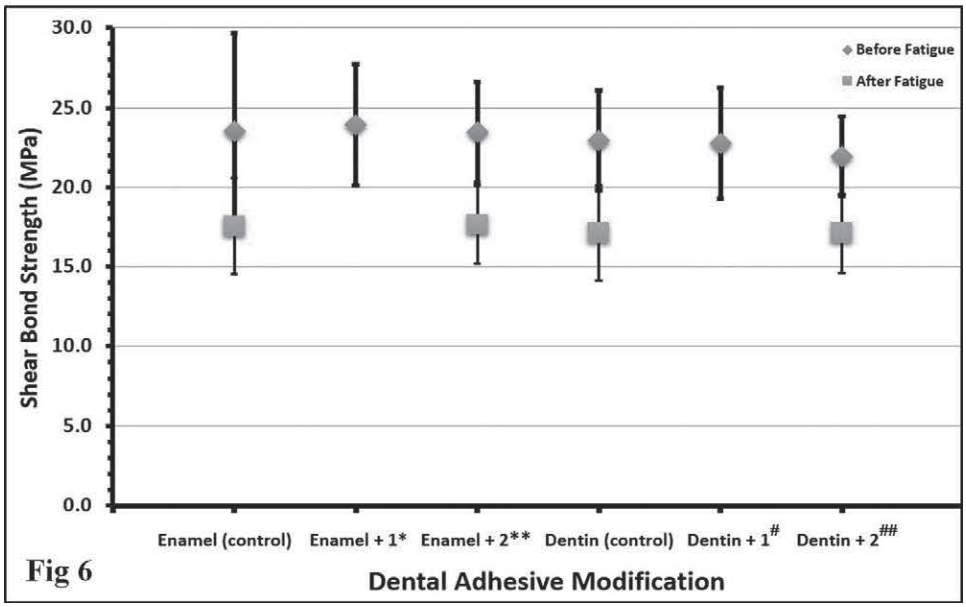


Figure 6. *Results of shear bond strength test. No statistically significant differences were determined between before-fatigue and after-fatigue groups,* p $>$ *0.05.* * *Bonding with D/E resin + 10 wt% MST;* # *bonding with D/E resin + 10 wt% nMT.* ** *Priming with D/E resin + 10 wt% MST;* ## *priming with D/E resin + 10 wt% nMT.*

reduced the titer counts, metabolic activity, and acid production of *Streptococcus mutans* biofilms.[22] Chlorhexidine (CHX) is also widely used as an antimicrobial agent, including for disinfection before placement of restorations. Hiraishi and others[23] reported that CHX had been shown to be released at relatively high rates from various methacrylate polymers. No inhibition effect on *Streptococcus*

Table 2: *Comparison of Experimental and Control Sections*

	Control		Experimental		Difference (Experimental−Control)				*p*-Value*
	Mean	SD	Mean	SD	Mean	SD	95% Confidence Interval		
Primer (n=10)									
Enamel	.77	.46	.80	.47	.03	.37	−.24	.29	.83
Dentin	2.18	.92	2.20	.89	.02	.47	−.31	.36	.88
Resin (n=10)									
Enamel	.67	.25	.72	.53	.04	.37	−.22	.30	.72
Dentin	2.60	.89	2.09	1.19	−.52	1.04	−1.26	.22	.15

* *Paired* t*-test on leakage measurements (average over three slices from each tooth).*

mutans was further detected from resin disks after two weeks of water storage. Matrix metalloproteinase (MMP) may be partially responsible for hybrid layer degradation. Loss of hybrid layer integrity compromised resin-dentin bond stability. CHX acted as an MMP inhibitor, so it had beneficial effects on the preservation of dentin bond strength.[23]

Our research is focused on gold NP (AuNP) since we discovered that gold-loaded microparticulate metal-titanate complexes (μMTs) were effective as a new class of antibacterials. Gold-loaded μMTs inhibit growth of oral bacteria at micromolar concentrations. Other studies[24] also demonstrated antifungal and antiviral actions of AuNP.

Compared to microparticles, NPs offer many advantages. Nanotechnology modulates metals into their nano-size, which drastically changes the chemical, physical, and optical properties of metals. Inorganic nanoparticles and their nanocomposites are applied as effective antibacterial agents. Nanoparticulate metal oxides have unusual crystal morphologies, with an increased number of edges and corners of NPs, which in turn generates a large NP surface area for interaction with bacteria. Theoretically, nMTs will be even more effective because they have a significantly greater surface-to-volume ratio, which is expected to lead to more effective ion-exchange characteristics. More importantly, they are able to act as drug carriers or to concentrate drugs on their surfaces, which results in polyvalent effects that enhance drug efficacy.[25-27] NPs themselves can specifically attack biological targets after modification with targeting molecules.[28,29] nMT also has the advantage of acting as a bioactive molecule to carry other drugs.

Oral applications of nanoparticles have recently been considered.[30] The potential of NPs to control oral biofilm formation is related to their biocidal and antiadhesive capabilities. NPs have been incorporated into dental materials to improve antimicrobial activity. The presence of antibacterials in both the bonding systems and the filling material theoretically would inhibit or slow both the initiation and progression of caries adjacent to restorations. Problems can arise as a result of release of the antibacterial agent from the composite. Such problems may include toxic effects, influence on mechanical properties, and loss of effectiveness.[31] If systemic toxicity could be limited and therapeutic indices were optimized, metal ions and their associated compounds could emerge as a new powerful class of antibacterial agents. Martínez-Gutierrez and others[32-3] and Kasraei and others[34] suggested that NPs may also exert significant cytotoxicity on macrophages in association with a proinflammatory response and cellular apoptosis. Their findings showed that silver nanoparticles were cytotoxic in murine macrophages and in fibroblasts at concentrations of 10 and 50 μg/mL, respectively. Instead of incorporating our nMTs into the body of the restorative materials, our strategy was to add the materials to the primer and adhesive layer. Such a strategy will minimize the quantity of materials needed. Moreover, amorphous peroxititanates (APT) might be used to bind a variety of metal compounds with high-affinity forming complexes to control the delivery of metal-based drugs to the target tissue, avoiding systemic toxicity. Wataha and others[4] demonstrated that metal-APT complexes facilitate metal ion delivery (such as gold and platinum) to monocytes as well as fibroblasts. Composite resins containing 1% silver nanoparticles exhibited antibacterial activity against *Streptococcus mutans* and *Lactobacillus*.[34] In addition, the presence of antibacterials in the bonding systems (ie, at the critical interface) would theoretically affect the initiation and progression of caries. Antibacterial adhesives could inhibit the invading bacteria along tooth-restoration margins.

Our attempt to incorporate the nMT into either the primer or the adhesive resin carries with it advantages and disadvantages. Aside from the pure chemical makeup point of view, putting the nMT into the primer will be closer to the tooth substrate and may have a more direct antibacterial effect. However, the nMT will be subsequently covered by bonding resin and composite resin restoration, and, thus, its long-term release may be hampered. Incorporating the nMT into the resin separates the active ingredient further from the targeted tooth surface. Both conditions are still at the critical exposed interface. Zhang and others[35] reported that adding dental resins containing 12-methacryloyloxydodecylpyridinium bromide (MDPB) with AgNP into both primer and adhesive achieved the strongest antibiofilm efficacy.

Our results showed that 10 wt% nMT, when added to the bonding agent, did not affect bond strength or microleakage for either enamel or dentin. Although cyclic loading did lower the after-fatigue SBS, when the groups are compared across the same conditions, we did not find any significant differences. Therefore, incorporating nMT into dental adhesive systems will not compromise resin-tooth bonding and sealing ability. Hence, the incorporation of antibacterial agents (ie, nMTs) into dentin bonding agents

may become an indispensable method for inhibiting residual bacteria in the cavity and secondary caries.

CONCLUSIONS

Within the limitations of this study, the addition of nMT to All-Bond 2® dental adhesive system did not affect the SBS and microleakage between composite and either human dentin or enamel. Incorporation of this novel material into a dental adhesive system does not appear to compromise either bonding or sealing ability and thus is a promising antibacterial agent with clinical significance.

Acknowledgements

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Regulatory Statement

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

Conflict of Interest

Author DCNC is one of the holders of the US patent No. US-2012-0156145-A1, "Use of titanium-based materials as bactericides," related to this manuscript. The other 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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Effect of Cigarette Smoke on Resin Composite Bond Strength to Enamel and Dentin Using Different Adhesive Systems

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Clinical Relevance

The exposure of dentin to cigarette smoke influences adhesive bonding strength. However, cigarette smoke does not influence the bond strength to enamel.

SUMMARY

Objective: To evaluate the microshear bond strength of composite resin restorations in dental blocks with or without exposure to cigarette smoke.

Method: Eighty bovine dental blocks were divided into eight groups (n=10) according to the type of adhesive (Scotchbond Multi-Purpose, 3M ESPE, St Paul, MN, USA [SBMP]; Single Bond 2, 3M ESPE [SB]; Clearfil SE Bond, Kuraray Medical Inc, Okayama, Japan [CSEB]; Single Bond Universal, 3M ESPE [SBU]) and exposure to smoke (no exposure; exposure for five days/20 cigarettes per day). The adhesive systems were applied to the tooth structure, and the blocks received a composite restoration made using a matrix of perforated pasta. Data were statistically analyzed using analysis of variance and Tukey test ($\alpha<0.05$).

Results: For enamel, there was no difference between the presence or absence of cigarette smoke ($p=0.1397$); however, there were differences among the adhesive systems ($p<0.001$). CSEB showed higher values and did not differ

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from SBU, but both were statistically different from SB. The SBMP showed intermediate values, while SB demonstrated lower values. For dentin, specimens subjected to cigarette smoke presented bond strength values that were lower when compared with those not exposed to smoke ($p<0.001$). For the groups without exposure to cigarette smoke, CSEB showed higher values, differing from SBMP. SB and SBU showed intermediary values. For the groups with exposure to cigarette smoke, SBU showed values that were higher and statistically different from SB and CSEB, which presented lower values of bond strength. SBMP demonstrated an intermediate value of bond strength.

Conclusion: The exposure of dentin to cigarette smoke influenced the bonding strength of adhesives, but no differences were noted in enamel.

INTRODUCTION

Of the development of restorative techniques in dentistry, adhesive systems have stood out for their ability to promote a bond between the tooth structure and restorative materials.[1,2] One way of classifying adhesive systems is related to the use of acid etching prior to the application of the adhesive (etch-and-rinse and self-etching). Although the current adhesive systems have been improved significantly after numerous studies, the interface between the substrate and adhesive is the susceptible failure when exposed to the oral environment. The tooth-restoration interface, called the "hybrid layer," is formed by a network of adhesive penetration into dentin tubules and enamel surface irregularities that becomes rigid when polymerized, allowing for the micromechanical retention of a resin restoration. The bonding durability between a restoration and tooth substrate is important for the longevity and clinical success of adhesive restorations.[3] Common clinical findings demonstrate that the exposure of this interface to the oral environment may cause the deterioration of the hybrid layer due to a variety of physical and chemical factors, including hydrolysis and enzymatic degradation of the dentin collagen.[4]

Cigarette smoke is composed of more than 5000 constituents, including carbon monoxide, ammonia, nickel, arsenic, and heavy metals such as lead and cadmium.[5-7] When cigarette smoke comes into contact with the tooth and restorations, it may cause discoloration, surface roughness, and hardness, which are considered important mechanical properties for clinical success of all restorations. Moreover, high temperatures (55°C) can change the properties of adhesive resins, such as sorption and solubility.[5,8] Furthermore, lead and cadmium are accumulated in teeth that are exposed to cigarette smoke, particularly on the enamel surface and within the dentin according to the exposure level,[5] decreasing the bond strength of resin-based restorative materials.[9] Therefore, smoking can affect the chemical and mechanical interaction between the composite resins and dental structures.[9,10]

It is not known to what extent the substances found in cigarette smoke may influence the adhesion of adhesive restorative materials to enamel and dentin and thus interfere with the durability and clinical success of these restorations. There are few studies in the literature that have evaluated the bond strength of different adhesive systems to dentin and the bond strength of resin-based restorative materials in enamel/dentin previously subjected to cigarette smoke. Therefore, the aim of this study is to verify whether cigarette smoke interferes with the adhesion between the dental substrates and various adhesive restorations. The null hypothesis of this study is that cigarette smoke does not affect the adhesion of system adhesives to dental structures.

METHODS AND MATERIALS

For this study, 80 bovine dental blocks were used and divided into eight groups (n=10; Table 1). Prior to the restoration process, half of the samples were exposed to cigarette smoke, as described below.

Specimen Preparation

The bovine incisors were collected, disinfected, and stored in a 0.1% thymol-buffered solution and distilled water. The crown was separated from the root using a double-faced diamond disc (KG Sorensen Ind. Com Ltda, Barueri, SP, Brazil). A metallographic cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) and diamond disc ($4 \times 012 \times$ 1/2 inches, Buehler) were used to obtain enamel/dentin blocks with a bonding surface area of 25 mm^2, 3 mm long and having a 1-mm enamel thickness. The enamel surface was ground using No. 600 and No. 1200 grit silicon carbide (SiC) abrasive papers under constant irrigation in a polishing machine (Arotec, Cotia, SP, Brazil). The specimens were then polished with felt disks (Arotec, Cotia, SP, Brazil) and diamond pastes (1, ½, and ¼ μm), with the specific lubricant (Arotec). Samples were placed in an ultrasonic tub (Marconi, Piracicaba, São Paulo, SP, Brazil) for 15 minutes between each application

Table 1: *Experimental Groups for the Adhesion Test in Enamel and Dentin*

Blocks Enamel and Dentin	System Adhesive	Exposure to Cigarette Smoke
SBMP (n=10)	Scotchbond Multi-Purpose (3M ESPE)	No
SB (n=10)	Single Bond 2 (3M ESPE)	No
CSEB (n=10)	Clearfil SE Bond (Kuraray)	No
SBU (n=10)	Single Bond Universal (3M ESPE)	No
SBMP-WS (n=10)	Scotchbond Multi-Purpose (3M ESPE)	Yes
SB-WS (n=10)	Single Bond 2 (3M ESPE)	Yes
CSEB-WS (n=10)	Clearfil SE Bond (Kuraray)	Yes
SBU-WS (n=10)	Single Bond Universal (3M ESPE)	Yes

Abbreviations: SBMP, Scotchbond Multi-Purpose; SB, Single Bond 2; CSEB, Clearfil SE Bond; SBU, Single Bond Universal; WS, with smoke.

of sandpaper and felt and at the end of the polishing procedures. All samples were stored in distilled water at 37°C until use.

Exposure to Cigarette Smoke

A smoke machine developed by the Department of Restorative Dentistry, Piracicaba Dental School UNICAMP, 2011 (registered under No. 01810012043 INPI, National Institute of Industrial Property) was used to expose groups SBMP-WS (Scotchbond Multi-Purpose, 3M ESPE, St Paul, MN, USA), SB-WS (Single Bond 2, 3M ESPE), CSEB-WS (Clearfil SE Bond, Kuraray Medical Inc, Okayama, Japan), and SBU-WS (Single Bond Universal, 3M ESPE; n=10) to cigarette smoke. The cycle was scheduled on a time interval, simulating the smoking behavior usually performed by a smoker, with the smoke remaining in contact with the specimens for three seconds. The machine allows for the ambient air to be inhaled every 10 seconds, thus simulating smoke inhalation and subsequent elimination. The specimens were subjected to smoke from one pack of Marlboro cigarettes (Philip Morris Brazil Ind. e Com, Santa Cruz do Sul, RS, Brazil) per day, for a total of five days.[11] In the interval between one simulation and another, the samples were stored in artificial saliva (1.5 mM Ca, 0.9 mM Pi, 150 mM KCL, 0.05 μg F/mL, 0.1 M Tris buffer [pH=7.0]) at 37°C, and every 24 hours, the samples were washed with distilled water and reimmersed in a fresh solution of artificial saliva to prevent sedimentation.[11,12] Prior to exposure of the samples to cigarette smoke, all samples were isolated with acid-resistant varnish (Colorama, São Paulo, Brazil), with the exception of the polished enamel area. The artificial saliva for all of the groups (exposed and not exposed to smoke) was changed daily.

Microshear Strength Test

Four types of adhesive systems were tested: 1) three-step etch-and-rinse adhesive system (Scotchbond Multi-Purpose [3M ESPE]), 2) two-step etch-and-rinse adhesive system (Single Bond 2 [3M ESPE]), 3) two-step self-etching system (Clearfil SE Bond [Kuraray Medical Inc]), and 4) one-step self-etching system (Single Bond Universal [3M ESPE]). These adhesive systems were applied to the tooth structure according to the manufacturer's recommendations (Table 2), and subsequently, the enamel blocks received a composite resin restoration (Filtek Z350XT Flowable; A3 shade, lot N495761, 3M ESPE) using a matrix of perforated pasta (Furadinho 6, Pastifício Santa Amália, Machado, Minas Gerais, Brazil) that was 1 mm in height and with a 1.15-mm internal diameter. This matrix does not cause tension during its removal after water absorption because the water gelatinizes the starch molecules and consequently reduces its rigidity. All enamel surfaces were etched using phosphoric acid 35% (Ultra Etch, Ultradent Products Inc, South Jordan, UT, USA) for 30 seconds. The photoactivation of the composite was performed using a third-generation LED source (Ultradent) at high-power mode, with a power density of 1400 mW/cm^2 for 20 seconds. The microshear test was carried out using the universal testing machine EZ Test-L (Shimadzu Corporation, Tokyo, Japan) at a speed of 0.5 mm/min. The microshear bond strength results were given in Mega Pascals (MPa) after measuring the bonding area using a digital caliper, according to the following formula:

$$R = \text{Rupture Force}(Kgf) \times 9.8/\text{Area}(\text{mm}^2)$$

where R is the bond strength in MPa.

Table 2: *Instructions for Use of Adhesives According to the Manufacturers*[a]

Adhesive System	Manufacturer's Instructions	Composition
Scotchbond Multi-Purpose (3M ESPE)	Etch enamel and dentin surface with phosphoric acid 35% for 30 and 15 s, respectively. Water rinsing twice as long the etching and dry carefully following the wet-bonding technique. Apply the *primer* to enamel and dentin and dry gently for 5 s (*no waiting*). Surface will appear shiny. Apply the adhesive to enamel and dentin and dry gently for 5 s. Light-curing for 10 s.	PRIMER: Water; 2-hydroxyethyl methacrylate (HEMA); copolymer of acrylic and itaconic acids BOND: Bisphenol a diglycidyl ether dimethacrylate (Bis-GMA) HEMA; triphenylantimony
Single Bond 2 (3M ESPE)	Etch enamel and dentin surface with phosphoric acid 35% for 30 and 15 s, respectively. Rinse for 10 s. Blot excess water using a cotton pellet or mini-sponge. **Do not air dry**. The surface should appear glistening without pooling of water. **Immediately after blotting**, apply two consecutive coats of adhesive for 15 s with gentle agitation using a fully saturated applicator. Gently air thin for 5 s to evaporate solvent. Light-curing for 10 s.	Ethyl alcohol; Bis-GMA; silane-treated silica (nanofiller); HEMA; copolymer of acrylic and itaconic acids; glycerol 1,3-dimethacrylate; water; diurethane dimethacrylate (UDMA); diphenyliodonium hexafluorophosphate; ethyl 4-dimethyl aminobenzoate (EDMAB)
Clearfil SE Bond (Kuraray)	Active application of the *primer for* 20 s. Air dry gently. Apply the *bond*, air dry gently, light-cure for 10 s. Etching enamel surface with phosphoric acid 35% for 30 s, previous application of adhesive.	PRIMER: 10-methacryloyloxydecyl dihydrogen phosphate hydrophilic aliphatic dimethacrylate; dl-camphorquinone (CQ); water BOND: 10-MDP, Bis-GMA, HEMA, CQ, hydrophobic dimethacrylate; N,N-diethanol P-toluidine; colloidal silica
Single Bond Universal (3M ESPE), self-etching method	Apply the adhesive for 20 s. Air dry gently for 5 s and light-cure for 10 s. Etch enamel surface with phosphoric acid 35% for 30 s, previous application of adhesive.	10-MDP; dimethacrylate resins; HEMA; Vitrebond copolymer; filler, ethanol; water; initiators; silane

[a] *As informed by the manufacturers' material safety data sheets.*

After the enamel microshear strength test, the enamel surface was removed to expose the dentin. SiC sandpaper, with a No. 600 granulation, was used to standardize the smear layer. The same protocol was used for the restorative procedures in dentin, and dentin microshear testing was performed. After the microshear test, bond failure mode was classified in percentages, as 1) cohesive in tooth tissue (enamel or dentin), 2) adhesive, 3) cohesive in the composite, and 4) mixed using a stereomicroscope (MZ75, Leica Microsystems, Heerbrugg, Switzerland) under 100×.

Statistical Analysis

The bond strength microshear data were subjected to analysis of variance (ANOVA) and Tukey tests ($\alpha<0.05$).

RESULTS

Table 3 presents the enamel microshear values. ANOVA showed significant differences for the factor adhesive system ($p<0.001$). However, no statistical difference was found for the factor exposure to cigarette smoke ($p=0.1397$) or for the interaction between the factors (cigarette smoke × adhesive

Table 3: *Mean (SD) Enamel Microshear Strength*[a]

Exposure to Cigarette Smoke	Adhesive System			
	Scotchbond Multipurpose (3M ESPE)	Single Bond 2 (3M ESPE)	Clearfil SE Bond (Kuraray)	Single Bond Universal (3M ESPE)
Without	17.55 (2.9)	12.75 (4.56)	19.00 (3.48)	19.09 (3.45)
With	14.30 (2.55)	11.17 (5.0)	19.23 (4.30)	18.72 (2.7)
Pooled data	15.93 (3.13) B	11.97 (4,73) C	19.12 (3.81) A	18.90 (3.01) AB

[a] *No effect of treatment (p=0.1397).Different letters indicate a significant difference (p≤0.05) between adhesive systems.*

Table 4: *Mean (SD) Microshear Strength in Dentin*[a]

Exposure to Cigarette Smoke	Adhesive System			
	Scotchbond Multipurpose (3M)	Single Bond 2 (3M)	Clearfil SE Bond (Kuraray)	Single Bond Universal (3M)
Without	14.60 (3.20) aB	15.24 (4.93) aAB	19.94 (4.45) aA	18.15 (4.49) aAB
With smoke	14.17 (5.05) aAB	10.16 (3.80) bB	9.97 (1.90) bB	18.82 (4.40) aA

[a] *Distinct letters (uppercase in the row and lowercase in the column) are statistically different (p<0.05).*

systems; $p>0.050$). Among these adhesive systems, CSEB showed higher values and did not differ from SBU, but both were statistically different from SB. SBMP showed intermediate values, whereas SB demonstrated the lowest values.

Table 4 presents the dentin microshear values. There was a significant difference between the factors exposure to cigarette smoke ($p<0.001$) and adhesive system ($p<0.001$) and for the interaction between the factors ($p<0.001$). For the groups without exposure to cigarette smoke, CSEB showed higher values that were statistically different from SBMP (lower values). SB and SBU showed intermediary values, without a significant difference between them. For the groups with exposure to cigarette smoke, SBU showed the highest values, which differed statistically from SB and CSEB, which presented the lowest values and did not differ statistically from each other. SBMP presented an intermediate value. When exposed to cigarette smoke, dentin showed lower values for SB and CSEB, which differed statistically from the groups that were not exposed to cigarette smoke. The data obtained in the fracture pattern evaluation were analyzed by frequency distribution (Figure 1). The mixed failure was the predominant pattern in almost all groups in enamel and dentin, with the exception for CSEB with exposure to cigarette smoke, which showed more adhesive failures.

DISCUSSION

In this study, there was no significant difference between the enamel groups with or without exposure to cigarette smoke, but there were differences between the adhesive systems (Table 3). The adhesion mechanism in enamel basically occurs through micromechanical retention from the infiltration of the adhesive system into enamel porosities that result from prior conditioning with phosphoric acid.[13] Based on this finding, it can be supposed that the incorporation of heavy metals did not affect the adhesion to enamel. The application of phosphoric acid for all groups provided demineralization of enamel, and contaminants may be removed from the enamel during this preparation, which could avoid bond degradation.

In relation to the adhesive systems for the enamel surface, CSEB showed the highest bond strength results. This agent contains functional monomers,

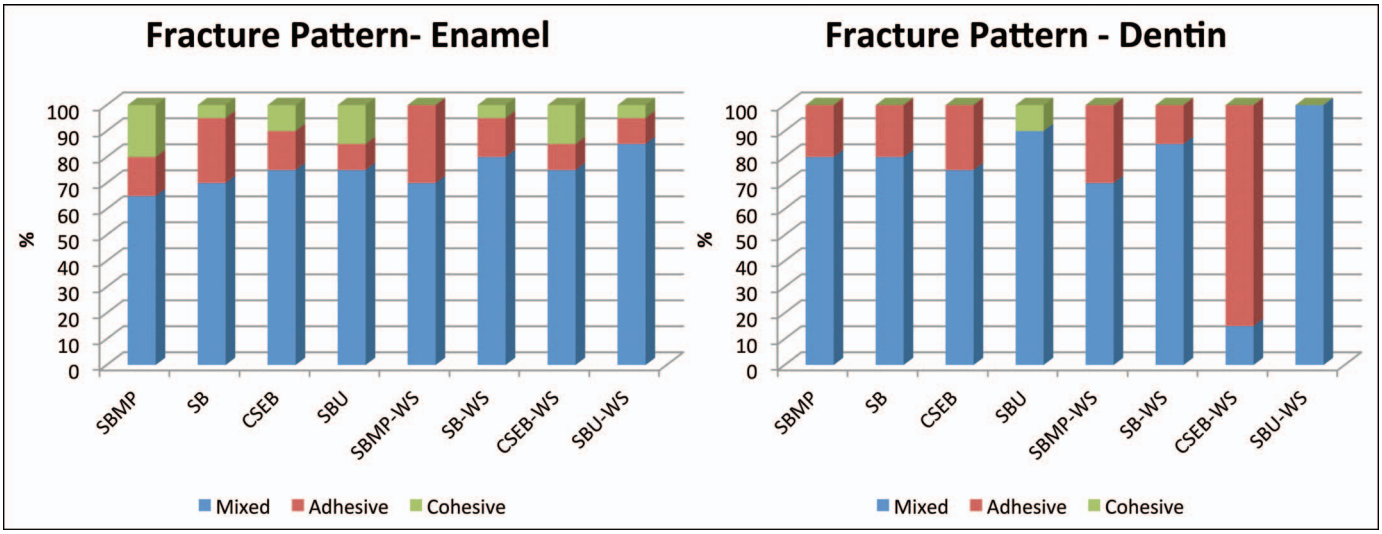

Figure 1. *Percentage of the failures: mixed, adhesive, and cohesive failure.*

which can establish chemical interactions between the adhesive and the hydroxyapatite in the dental substrate.[14] The commonly used functional monomer in this adhesive is the phosphate monomer, 10-methacryloxydecyl dihydrogen phosphate (10-MDP). The AD–Concept can explain the results for CSEB, for which phosphate-based monomers (such as phenyl-P and 10-MDP, which are part of the acidic primer) have a potential for chemical bonding with calcium in hydroxyapatite. Therefore, all of the acids interact with the calcium in the hydroxyapatite, forming ionic bonds that are stable.[14] Moreover, etching of the enamel surface removes the smear layer and increases the reactivity of 10-MDP with the calcium from hydroxyapatite, thus improving the bond strength.[15]

SBU presented intermediate values, without a significant difference from the other groups. SBU is a one-step self-etching adhesive; this adhesive category presents complex mixtures of hydrophilic and hydrophobic components to produce thinner hybrid layers when compared with two-step and etch-and-rinse adhesives.[16-18] SBU also contains functional monomers and a Vitrebond copolymer. Both compounds interact with the calcium from hydroxyapatite.[19] Previous studies[20,21] demonstrated that etching the enamel significantly increased bond strength values for the one-step multimode adhesive, SBU, and the two-step self-etching adhesive, CSE.

SB presented the lowest bonding values to enamel, and SBMP presented intermediate values, without any significant differences from SBU. SB is classified as a two-step etch-and rinse adhesive because the primer (part hydrophilic) and bond (part hydrophobic) are in the same bottle[2,22] and because the solvent is mostly water (enamel contains only 4% water). The infiltration of the two-step etch-and rinse adhesive system is lower when compared with three-step etch-and rinse adhesives. The three-step etch-and rinse adhesives contain hydrophilic functional monomers in the primer, which allow for the monomer to permeate into the demineralized matrix, while the hydrophobic monomers contained in the bonding agent facilitate adhesion of the composite restorative material to the conditioned tooth surface.[23] Moreover, SB showed problematic solvent evaporation,[24] and the presence of the solvent in the adhesive layer decreased the degree of conversion,[24] which consequently provided a lower bond strength.

When considering the dentin surface, there was a statistical difference between some groups with and without exposure to cigarette smoke, with SB-WS and CSEB-WS presenting lower bond strength values when compared with their respective smokeless groups (Table 4). It can be suggested that the heavy metal contaminants interfered with the chemical interaction between the functional monomers 10-MDP and the Ca hydroxyapatite, the main bonding mechanism for CSEB-WS. There were no statistical differences between the groups with or without exposure to cigarette smoke for the SBU group, which may indicate that there are other monomers in addition to 10-MDP, including the Vitrebond copolymer, that might have the chemical property to interact with the tooth structure, since this adhesive demonstrated better results. However, more studies are necessary to verify this interaction, because there are no studies in the current literature.

SB also showed a lower bond strength for the WS groups. This result was expected: the total etching would remove the mostly heavy metals incorporated from cigarette smoke. However, the presence of heavy metals in an adhesive with problematic solvent evaporation could inhibit the degree of conversion and bond strength of this adhesive. This mechanism did not occur with the three-step etch-and-rinse adhesives, since the primer adhesive system (hydrophilic) and bond (hydrophobic) are in different bottles.[2,22]

Only SBU maintained the same bond strength when exposed to cigarette smoke when comparing the adhesive systems that showed higher bond strengths in dentin without smoke (SB, CSEB, and SBU). SBMP presented a lower bond strength in dentin without smoke; however, this system also maintained the bond strength for the group with smoke exposure. Almeida e Silva and others[9] indicated that contamination by cigarette smoke decreased the bond strength between dentin and composite resin because of the reduced diameter of the particles from cigarette smoke, which are capable of being absorbed into the dentin hydroxyapatite based on the exposure level,[5] reducing the contact between the adhesive and dentin and thus reducing the bond strength values.[3,5] After acid etching, it can be inferred that heavy metals may remain in dentin, even after washing the surface, which can damage simplified adhesive systems.

The null hypothesis of this study was partially accepted: cigarette smoke did not affect the adhesion to enamel but reduced the adhesion to dentin for the SB and CSEB adhesive systems.

CONCLUSION

The exposure of cigarette smoke influenced the bonding strength of some adhesives to dentin, but no changes were observed for enamel.

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Conflict of Interest
The authors 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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Longevity of Bonding of Self-adhesive Resin Cement to Dentin

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Clinical Relevance

Regardless of the cementing strategy, the durability of bonding to root canal dentin may be influenced by the dentin treatment protocol.

SUMMARY

Objective: To evaluate the effect of root dentin treatment on the bonding of self-adhesive resin cement after 24 hours and after 6 months.

Methods: A total of 48 single-rooted premolars were endodontically treated and divided into four groups (n=12): Adper Scotchbond Multi-Purpose + RelyX ARC (ARC); RelyX U200 (U200); EDTA + RelyX U200 (EU200); and phosphoric acid (H_3PO_4) + RelyX U200 (HU200). After filling the roots, an Exacto No. 2 fiber post was cleaned, treated with silane (60 seconds), positioned, and light cured (LED; 60 seconds at 1200 mW/cm^2). After storage (37°C/24 h), the roots were cut to obtain two discs (1 mm) of each third. They were stored in distilled water (24 hours at 37°C); one disc of each root-third was subjected to the push-out test (0.5 mm/min) at 24 hours and the other disc after six months of water storage (37°C). The data on the root-thirds were averaged for statistical purposes. The average values of bond strength (MPa) were analyzed by two-way analysis of variance and post hoc Student-Newman-Keuls (5%).

Results: There were statistical differences for the treatment of dentin ($p<0.001$), for time ($p=0.003$), and the interaction of treatment and time ($p=0.017$). After 24 hours, we observed lower bond strength in the HU200 group when compared with other groups (ARC, U200, and EU200). After six months, HU200 showed the lowest bond strength. Higher strengths were observed for EU200 and U200 similarly, which were higher than ARC.

Conclusion: The bonding of the self-adhesive resin cement varied over time in the tested groups.

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INTRODUCTION

Fiber posts have several properties (eg, esthetics and elastic modulus) that are similar to those of tooth structure, which provides excellent biomechanical behavior and better load dissipation, reducing the risk of radicular fracture.[1,2] A post's association with the adhesive system and resin cement improves the performance of their mechanical properties.[3,4]

Resin cements are recommended for cementing the fiberglass posts in the root canal and are divided into self-adhesive and adhesive systems according to the cementing strategy.[5] Self-adhesive cement was developed a decade ago, with the purpose of simplifying the cementation process by assembling all the components into a single product.[6] This combination has resulted in a material that self-adheres to dentin, does not require pretreatment of the surface of the tooth, is simple to implement, and can be performed in a single step.[5,7] Given that the removal of the smear layer is not recommended with most self-adhesive cements, there is increased tolerance to moisture and the release of fluoride ions.[5]

Some studies have indicated that retaining the smear layer on the dentin could interfere with the adhesion of self-adhesive materials because it may hinder the adaptation and bonding of the resinous material to the walls of the root canal.[8,9] Others have indicated its retention because some solutions used to remove it could modify the structure of the dentin, increase water flow, and compromise the bonding with the resinous monomers by interfering with the polymerization.[10]

There is no consensus in the literature regarding the treatment of dentin in preparation for self-adhesive resin cement. Some studies have suggested that the cement is able to cross the smear layer and bond to the dentin.[5-7] The simplification of the cementation technique has been welcomed by clinics, and according to a recent study[11] has demonstrated bonding similar to the resin cement associated with the adhesive system because the latter system is more sensitive due to the various steps involved and can incur operator errors.[12] However, to our knowledge, the durability of the bond strength of self-adhesive resin cements is still unknown.

This investigation was conducted to test the hypotheses that 1) there is no difference between treating the dentin with ethylenediaminetetraacetic acid (EDTA) at a concentration of 17% or phosphoric acid (H_3PO_4) at a concentration of 35% in the strength of the push-out bond strength to dentin when resin cement is applied; 2) there is no difference at 24 hours and after six months in the strength of the push-out bond strength to dentin when resin cement is applied.

METHODS AND MATERIALS

A total of 48 single-rooted human premolars, with complete root apex formation, that were free of caries, fractures, root lacerations, and previous endodontic treatment and had at least 14 ± 1 mm between the cementoenamel junction (CEJ) and the root apex were disinfected in a 0.5% solution of chloramine-T for seven days at 4°C.

Preparation of the Teeth

The dental crowns were sectioned in a direction perpendicular to the long axis at the height of the CEJ to obtain roots of a minimum length of 14 mm and to create access to the root canal. The patent canal length was the established working limit and the instrumentation was performed up to the apical foramen. The roots were filled using the hybrid trigger technique, maintaining a distance of 1 mm from the apex with the endodontic cement, Sealer 26 (Dentsply, Dentsply DeTrey, Konstanz, Germany). The cones were condensed using a McSpadden device (Easy Dental Equipment, Belo Horizonte, Brazil). After allowing for the cement setting time, the 48 roots were unobstructed using the equipment Termo Pack II (Easy Dental Equipment, São Paulo, Brazil). The specimens were wrapped in sterile gauze soaked in a solution of 0.9% sodium chloride (NaCl), packed in individual containers, and stored for 24 hours at room temperature. For the final calibration of the conduit a No. 2 Exacto drill (1.6 mm in diameter; Angelus, Londrina, Brazil) was used, coupled to a low-speed turbine (Koncept, KaVo, Joinville, Brazil) to a maximum depth of 9 mm from the CEJ.

Experimental Groups

The composition and application mode of the materials used are presented in Table 1. The prepared roots were irrigated with a solution of 0.9% NaCl and dried with absorbent paper towels before being categorized from one to 48 and randomly allocated to four treatment groups (n=12). Each specimen received a translucent fiberglass post Exacto No. 2 (Angelus) with a conical shape, smooth surface, diameter greater than 1.6 mm, and length of 15 mm. Its surface was cleaned with sterile gauze soaked in absolute ethanol (F Maia, Cotia, Brazil) and dried with jets of air. The binding agent, Silane

Table 1: *Composition and Application Mode of the Materials Used in the Study*

Material / Manufacturer	Composition	Application
Ethylenediaminetetraacetic acid (EDTA) / Biodinâmica	17% aqueous solution of tetrasodium salt (1 M; pH 7.2)	Apply with 5 mL on the surface (3 min), Wash (0.9% NaCl for 30 s), dry with absorbent paper points.
Phosphoric acid conditioner Scotchbond / 3M ESPE	35% phosphoric acid	Apply (15 s) on dentin, wash with water (30 s), dry with absorbent paper points.
Silane / Angelus	monofunctional γ-methacryloxypropyltrimethoxysilane (MPS) and ethanol	Apply for 60 s and lightly air dry.
RelyX U200 Automix / 3M ESPE	Paste A—amine, bisphenol A glycidyl methacrylate (bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), photoinitiators, inorganic particles of silica and zirconia (68% by weight), and pigments.	Apply (Automix syringe), wait the setting time.
	Paste B—TEGDMA, bis-GMA, inorganic particles of silica and zirconia (67% by weight), benzoyl peroxide.	
RelyX ARC / 3M ESPE	Silane-treated ceramic, TEGDMA, bis-GMA, silane-treated silica, functionalized dimethacrylate polymer.	Mix equal parts of Paste A and Paste B (10 s)
		Apply (Centrix syringe), wait the setting time
ScotchBond Multi-Purpose Plus (SBMP) / 3M ESPE	35% phosphoric acid	Acid etching (15 s); wash (30 s); dry (absorbent paper); apply activator (one coat); apply primer; apply catalyst
	SBMP activator: sulfinic acid salt ethanol based solution, photoinitiators	
	Primer: 2-hydroxyethylmethacrylate (HEMA), polyalkenoic acid copolymer	
	SBMP catalyst: bis-GMA, HEMA, benzoyl peroxide	
Fiberglass post Exacto / Angelus	80% glass fiber	Place the post in the root
	20% epoxy resin	

(Angelus), was applied for 60 seconds with an extra-fine disposable brush (KGbrush, KG Sorensen, Barueri, Brazil), and the excess removed with jets of air.

The root dentin was conditioned according to experimental group as follows: ARC group, 35% H_3PO_4 gel (3M ESPE, St Paul, MN, USA) for 15 seconds; U200 group, not conditioned; EU200 group, conditioned with a solution of 17% EDTA (Biodinâmica, Ibiporã, Brazil) for three minutes; and the HU200 group, conditioned with 35% H_3PO_4 for 15 seconds.

Following this, the root dentin was washed with water for 30 seconds by means of a triple syringe and dried with absorbent paper, leaving the canal slightly damp. In the ARC group, the posts were cemented after application of the adhesive system Adper Scotchbond Multi-Purpose (3M ESPE) with resin cement RelyX ARC (3M ESPE) by means of a Centrix syringe (Nova DFL, Rio de Janeiro, Brazil). In the U200, EU200, and HU200 groups, the posts were cemented with ready-mixed self-adhesive resin cement, RelyX U200 Automix (3M ESPE), inserted directly into the canal. In all groups, the cements were light-cured using a LED device (Radii-Call, SDI, Bayswater, Australia) with an irradiance of 1200 mW/cm^2 for 60 seconds from the coronal direction.

After preparation of the 48 specimens according to their experimental group, the specimens were stored for 24 hours in humidified individualized containers in an oven at 37°C. With the aid of a diamond-cutting disc (Extec 12205, Erios, São Paulo, Brazil) coupled to a cutting machine (model Isomet 1000, Buhler Ltd, Lake Bluff, IL, USA) at a speed of 200 rpm under constant cooling with distilled water, two 1-mm thick slices of each root-third of the specimens were obtained (which means that six discs were obtained from each root). They were stored in distilled water (37°C/24h). At 24 hours, three discs from each root (cervical, medial, apical) were subjected to a push-out test, and the other three discs from each root (cervical, medial, apical) were kept stored in distilled water at 37°C to be tested

Table 2: *Mean (Standard Deviation)in MPa of Bond Strength to Dentin According to the Experimental Conditions*[a]

Time	Surface Treatment				
	ARC	U200	EU200	HU200	*p* value
24 h	4.99 (1.79) Aa	5.04 (1.36) Aa	3.84 (1.64) Aa	2.38 (1.63) Bc	0.001
6 mo	4.54 (2.40) Aa	6.79 (2.41) Ab	7.11 (1.83) Cb	3.19 (2.48) Bc	
p value	0.03				

[a] *Lowercase letters show comparison inside the rows whereas uppercase letters indicate comparisons among columns.*

after six months. The storage medium was changed weekly.[13]

Mechanical Testing

Each disc was fixed in a device and a compression load applied to the slice in the apical-coronal direction so as to push the post, respecting the taper of the root canal, by means of a 1-mm-diameter cylindrical punch connected to a universal testing machine (Emic DL 2000, São José dos Pinhais, Brazil) at a speed of 0.5 mm per minute, using a load cell of 50 kgf until the post was displaced in the root canal. The bond strength was obtained in newtons and converted to MPa by dividing the maximum load failure obtained by the area of the bonded interface. After being subjected to the push-out test, the dimensions of the specimen were measured using a digital caliper, Digimess (Digimess Precision, São Paulo, Brazil), with an accuracy range of 0.02 mm.

Analysis of Fracture Pattern

The failure surfaces were examined with an optical microscope (Bel MicroImage Analyzer, Bel Photonics, Monza, Italy) with a magnification of 40× to determine the type of failure, categorized as follows: 1) adhesive failure between the post and cement, 2) adhesive failure between the cement and root dentin, 3) cohesive failure of the post system, 4) cohesive failure of the cement, and 5) mixed type, a combination of the two aforementioned failures. Representative specimens were selected and analyzed with a scanning electron microscope (SEM; SSX 550 EDX, Shimadzu, Bangkok, Thailand).

Dentin Micromorphology

The same SEM was used to characterize the root dentin surface. After preparation of the teeth in the same way as previously described, each root was cut in the direction of the long axis of the buccal and lingual surfaces of each root with the aid of a diamond disc at a speed of 200 rpm under constant cooling with distilled water (Extec 12205, Erios) coupled to a cutting machine (model Isomet 1000, Buhler Ltd). Following that, the conditioning protocols of each experimental group were carried out; next, the roots were broken at the end to obtain two pieces of each root, which were then fixed with paraffin in aluminum stubs and left in a dry oven with silica for 24 hours to lose all moisture. Next, they received gold metallization in a vacuum chamber (Sputtering SCD050, Bal-Tec, Balzers, Liechtenstein) and were taken to the SEM for observation at 12 kV operating in secondary electron mode. Images of low (30×) and high (1200×) amplitude were obtained of the coronal, middle, and apical thirds of each root, and each one was qualitatively evaluated.

Statistical Analysis

For statistical purposes, the data of the root-thirds were grouped and a root considered as the experimental unit.[12,14] The mean values of bond strength of the roots were transformed into square roots and subjected to a two-way analysis of variance (Treatment × Time). All pairwise multiple comparisons were performed using the Student-Newman-Keuls method with a significance level of $\alpha = 0.05$. The statistical software Sigma-Stat 3.5 (San Jose, CA, USA) was used.

RESULTS

Table 2 presents the mean values of bond strength in the experimental groups. A statistically significant difference was observed for the treatment of the root dentin ($p < 0.001$; power of test = 1), for the time ($p = 0.003$; power of test = 0.83), and for the interaction of treatment and time ($p = 0.017$; power of test = 0.609). The results demonstrated that conditioning the dentin with 35% H_3PO_4 statistically decreased the bond strength at 24 hours when compared with U200, EU200, or ARC. With regard to the six-month results, HU200 showed the lowest bond strength. Higher bond strength was observed for U200 and EU200 similarly, which was higher than ARC.

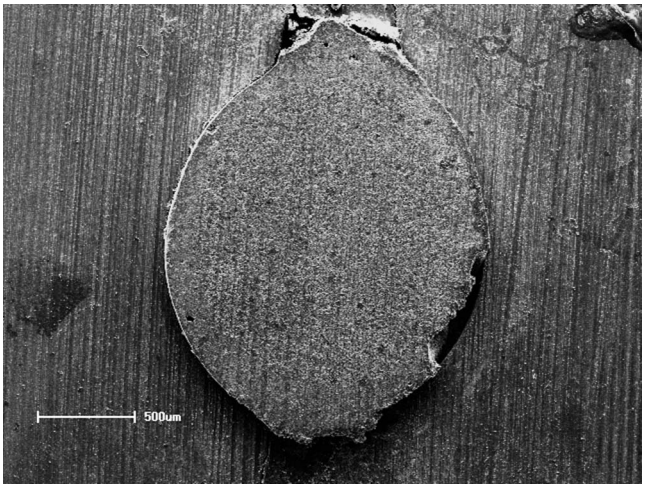

Figure 1. *Scanning electron microscopy of adhesive fracture mode.*

Observation of the fracture pattern showed the predominance of adhesive failure between the cement and root dentin in all the experimental groups, followed by a lower rate of adhesive failure between the cement and root. There was no cohesive failure reported in the present study (Figure 3). A representative image of the predominant failure mode can be seen in Figure 1.

Figure 2 presents SEM imagery of the root dentin treated with acidic solutions, in which the root covered by the smear layer in the coronal (A), middle (B), and apical (C) thirds in the group that did not receive acid conditioning is noted. After conditioning with 17% EDTA (coronal [D], middle [E], apical [F]) and 35% H_3PO_4 (coronal [G], middle [H], apical [I]), there was exposure of the dentinal tubules.

DISCUSSION

The objective of this study was to evaluate the influence of root dentin treatment prior to cementing a fiberglass post with self-adhesive resin cement and a resin cement associated with the adhesive system after 24 hours and after six months. The results showed that the bonding between the dentin and the cement was influenced by the dentin treatment and the storage time.

After 24 hours, the adhesion of the U200 was similar to that of the EU200 and ARC, and all three were greater than the HU200, rejecting the first hypothesis of this study. The results corroborate a systematic review[11] that observed similar adhesion between resin cements, regardless of the cementing strategy. The similarity could be explained by the conditioning of the dentin having been performed with a weak acid such as 17% EDTA, which superficially exposed the dentinal tubules[9,10] of the root canal (Figure 2) and enabled the interaction of the self-adhesive cement with the dentin underlying the smear layer. Promising results were reported after the etching of the dentin with EDTA[15-17] by greater unobstruction of the dentinal tubules and the formation of the hybrid layer.

Despite the self-adhesive strategy theoretically eliminating the need for any surface treatment of the dentin, there are doubts about retaining the smear layer, which, according to some authors,[16,18] could interfere with the bonding to the root canal, and therefore propose its removal with chemical solutions such as polyacrylic acid, EDTA, NaCl, and sodium hypochlorite (NaOCl). However, one review[9] showed that the removal of the smear layer has not been standardized among different studies and cannot be removed uniformly along the full extent of the root canal, given that switching solutions of 17% EDTA with 5.25% NaOCl led to greater removal of the smear layer. Figure 2 shows the removal of the smear layer and unobstruction of the dentinal tubules by 17% EDTA, partially agreeing with the results of this review, because in this study EDTA was not switched with another substance. Conditioning of the dentin with 35% H_3PO_4 also demonstrated a similar result.

This study also showed that the etching of the dentin with 35% H_3PO_4 resulted in lower bond strength after 24 hours and after six months. But when the adhesive system was applied after etching with H_3PO_4 as in the ARC, the formation of the hybrid layer ensured bonding similar to the U200. Studies within the scope of the adhesive cementation of posts usually use the ARC as a comparison group[19] and promising results have been observed; however, few of these assessed bond strength after 24 hours.[20]

Thus, the second objective of this investigation was to evaluate the longevity of bonding of the resin cement/dentin after six months, and the results showed that the storage time influenced the adhesion, rejecting the second hypothesis of this study. After six months, the adhesion of the EU200 group increased, and it was higher than that of all the other groups. These results can be explained by the use of EDTA, a molecule containing four carboxylic acid groups, which acts as a light chelator of calcium (Ca) in selective dissolution of hydroxyapatite.[21] Unlike phosphoric acid etching, which is deep, with EDTA the dentin is superficially demineralized.[22] It can be understood that the adhesion of the EU200 group was higher than the ARC at six months due to the interaction of the resin cement and the dentin, with

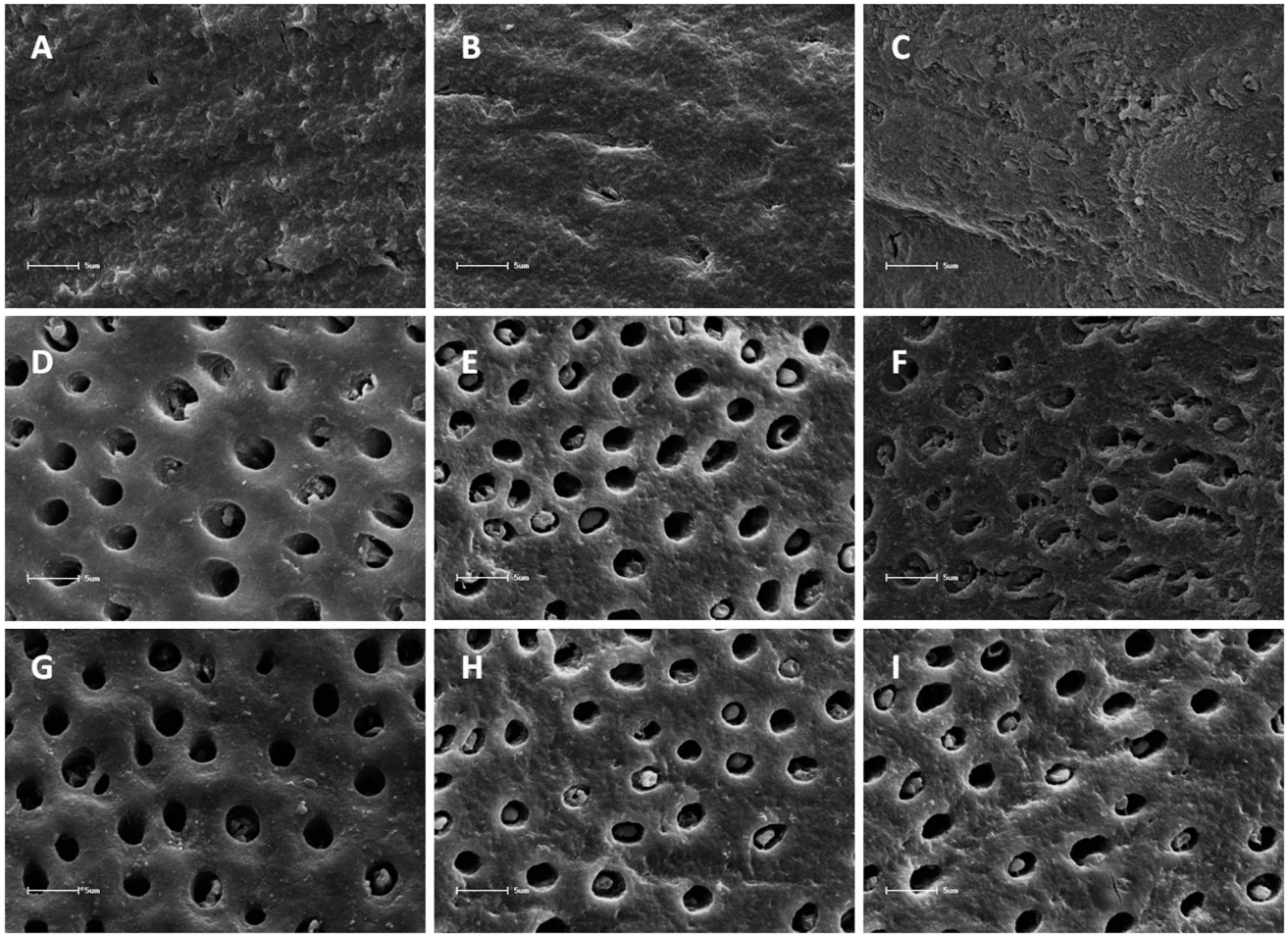

Figure 2. *Scanning electron microscopy of radicular dentin after surface treatments. Without acid etching: (A) cervical, (B) middle, (C) apical; After etching with 17% EDTA: (D) cervical, (E) middle, (F) apical; After etching with 35% H_3PO_4 (G) cervical, (H) middle, (I) apical.*

the latter having been more uniformly demineralized by the EDTA compared with the HU200 group.

The preservation of the longevity of adhesive restorations was described when the conditioning of the dentin with EDTA was carried out,[22,23] where surface removal of the hydroxyapatite ensured that the collagen fibers would not be exposed or suffer structural changes[24] or dehydration.[25] Another aspect to be considered is that EDTA is a potent inhibitor of matrix metalloproteinases (MMPs), collagenolytic enzymes related to the degradation of the organic matrix.[24,26] The chelating activity of EDTA promotes the sequestration of Ca^{+} ions present in the dentin,[21] which together with zinc, act as potential activators of MMPs.[26,27] When performing the conditioning with phosphoric acid etching, greater demineralization and exposure of collagen occurs, activating MMPs.[24] These aspects may also explain the results observed at six months in this study.

The bonding of resin cement to dentin will occur if there is an interaction between the surfaces.[28] Although the pH of some self-adhesive cements is initially acidic, it is unable to completely remove the smear layer as a strong acid such as H_3PO_4 would.[7,29,30] For some authors[31] this limitation could be overcome by the possible chemical bond between the dentin and some self-adhesive resin cements that contain the functional monomer. In the case of the U200, according to the manufacturer, the chemical adhesion is ensured by the presence of 4-methacryloxyethyl trimellitic acid monomers and phosphoric acid esters.

Conditioning the root dentin with H_3PO_4 before cementation seems to hinder adhesion to the dentin[4,7,30] by exposing the collagen, deeply removing the hydroxyapatite, and changing the water flow.[32,33] The results of the HU200 group in this study agree with these assertions. However, the application of the adhesive system after conditioning

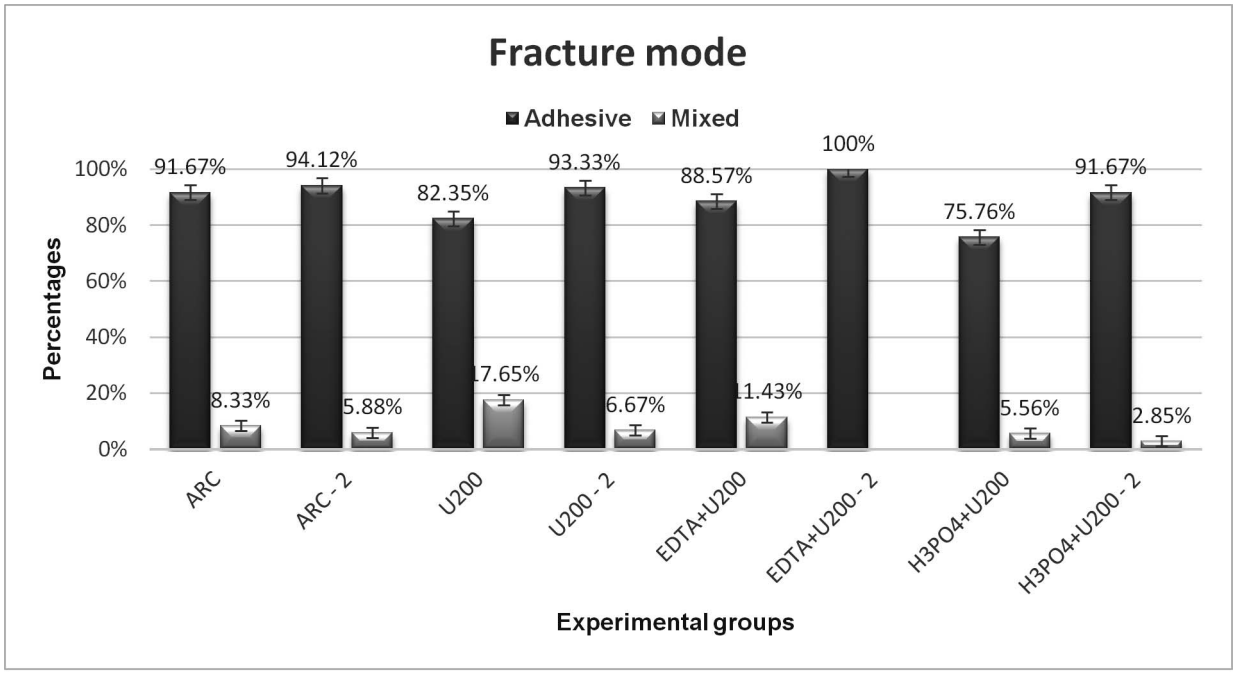

Figure 3. *Percentages of fracture modes according to the experimental conditions.*

with this acid ensured adhesion in the ARC group after 24 hours and after six months. Thus, the result of this study demonstrated that phosphoric acid should not be used in isolation as a dentin conditioner, prior to the self-adhesive cement studied, because RelyX U200 contains hydrophilic monomers that can interfere with the cement polymerization process, compromising the bonding.[17]

The adhesion of the fiberglass post to the root dentin can be influenced by the difficulty in controlling the dentin moisture when used with a resin cement adhesive system, by visualizing the canal along its entire length,[29,33] and by the formation of bubbles in the cement interfaces.[34] In the present study this aspect was minimized because the RelyX U200 cement was inserted with the adapted tips that accompanied the Automix kit (3M ESPE) and for the RelyX ARC cement a Centrix syringe (Nova DFL) was used. This detail of the methodology and the silanization of the fiberglass posts favored the prevalence of adhesive fractures between the cement and root dentin, agreeing with previous studies that found the majority of fractures in the same place[33,34] and disagreeing with a study that observed a predominance of fractures in the cement-post interface.[32]

According to Heintze and Zimmerli,[35] the push-out test is commonly used to analyze the post adhesion of roots, and well-designed and conducted *in vitro* bond strength tests are useful as a screening test if the specimens are tested after one day and after at least three months of water storage. In this study, the bonding of fiber posts to dentin was analyzed after 24 hours and after six months of water storage, more than suggested in the literature.[35] The present investigation analyzed the bonding of RelyX U200, a self-adhesive resin cement, comparatively with that of RelyX ARC, a gold standard used on cementation of fiber posts to dentin, using natural teeth, which gives results close to the clinical outcome.[36] Further research on self-adhesive cementation is needed, especially randomized controlled clinical trials.

CONCLUSIONS

Given the limitations of this study, it may be concluded that the bonding durability of resin cements to root dentin varied over time, according to the surface treatment of dentin.

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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 Ethics Committee of Unopar. The approval code for this study is 95060.

Conflict of Interest

The authors 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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Composite Replacement of Amalgam Restoration Versus Freshly Cut Dentin: An *In Vitro* Microleakage Comparison

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M Finkelman • S Khayat • H-P Weber

Clinical Relevance

Bonding to dentin under replaced amalgam restorations may be as effective as bonding to freshly cut dentin.

SUMMARY

Objective: The aim of this study was to evaluate the microleakage of the composite restorations when bonded to tooth structure previously restored with amalgam material compared with that of freshly cut dentin.

Methods and Materials: Thirty intact, extracted intact human molars were mounted in autopolymerizing acrylic resin. Class II box preparations were prepared on the occluso-proximal surfaces of each tooth (4-mm bucco-lingual width and 2-mm mesio-distal depth) with the gingival cavosurface margin 1 mm above the CEJ. Each cavity was then restored using high copper amalgam restoration (Disperalloy, Dentsply) and then thermocycled for 10,000 thermal cycles. Twenty-five of the amalgam restorations were then carefully removed and replaced with Filtek Supreme Ultra Universal (3M ESPE); the remaining five were used for scanning electron microscopy and energy dispersive x-ray spectroscopy analysis. A preparation of the same dimensions was performed on the opposite surface of the tooth and restored with composite resin and thermocycled for 5000 thermal cycles. Twenty samples were randomly selected for dye penetration testing using silver nitrate staining to detect the microleakage. The specimens were analyzed with a stereomicroscope at a magnification of 20×. All of the measurements were done in micrometers; two readings were taken

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for each cavity at the occlusal and proximal margins. Two measurements were taken using a 0-3 scale and the percentage measurements.

Results: Corrosion products were not detected in either group (fresh cut dentin and teeth previously restored with amalgam). No statistically significant difference was found between the microleakage of the two groups using a 0-3 scale at the occlusal margins (McNemar test, $p=0.727$) or proximal margins (Wilcoxon signed-rank test, $p=0.174$). No significance difference was found between the two groups using the percentage measurements and a Wilcoxon signed-rank test at either the occlusal ($p=0.675$) or proximal ($p=0.513$) margins. However, marginal microleakage was statistically significant between the proximal and occlusal margins ($p<0.001$).

Conclusion: Within the limitations of this *in vitro* study, no significant difference was found between the microleakage of nondiscolored dentin in teeth that were previously restored with amalgam compared with freshly cut dentin. However, marginal microleakage in the proximal surface was higher than that in the occlusal surface.

INTRODUCTION

The use of composite resin material has increased and has become the first choice for most carious lesions. This change can be attributed to several factors.[1] Some of the main factors are that amalgam restorations do not adhere to the cavity wall chemically, and there are critical differences in the coefficient of thermal expansion of amalgam and tooth structure. Amalgams allow the transport of fluids, ions, molecules, and possibly bacteria and their toxins more readily.[2,3] These problems are more obvious when poor operative technique is used. Moreover, mercury used in amalgams has raised concerns about its biological toxicity and environmental hazards. People are exposed to mercury and other metals via vapor and corrosion products in swallowed saliva and by direct absorption through the oral mucosa.[4-6] However, appearance, in addition to the controversy about mercury toxicity, is the public's displeasure with amalgam. Additionally, amalgam has other disadvantages, in that corrosion can cause increased porosity and reduced marginal integrity and strength, as well as the release of metallic products into the oral cavity.[4] The literature supports a lack of evidence that amalgam and its mercury content can be harmful to humans, with the only exception being specific metal allergies.[7]

The use of tooth-colored restorative material rather than amalgam restorations requires the meticulous use of an adhesive technique.[8] This is because dentin is more hydrophilic in nature compared with enamel and therefore is more difficult to deal with in terms of bonding to adhesive resin. However, enamel is known to have reliable bond strength when bonded to resin-based composites, because its main components are inorganic, whereas dentin has a different composition and structure.[9,10]

Although various generations of bonding agents have been developed to decrease polymerization shrinkage, microleakage remains a significant problem[11] and a major factor that affects the longevity of composite restorations.[12] This is a primary cause of marginal discoloration, secondary caries, pulpal inflammation, postoperative sensitivity, and restoration replacement.[13] Microleakage measurements usually provide an assessment of the sealing ability of adhesive materials, which is clinically relevant.[14]

According to Abo and others,[15] an *in vitro* microleakage test combined with thermocycling is a useful method to assess sealing performance. In their study, they also demonstrated that the use of a large number of thermal cycles could simulate the conditions in the oral environment. In a review performed by Heintze[16] on the evaluation of in vitro sealing ability, microleakage testing was preferred over other methods because of its ease and simplicity. The second most frequent method, the quantitative marginal analysis of replicas using scanning electron microscopy (SEM) is used less often because it is more time consuming and complex[16]

In many clinical situations, adhesive resin is used to bond to sclerotic dentin or dentin discolored by corrosion products from the amalgam restorations that have penetrated the dentinal tubules. Several studies have shown that the bond strength was lower than or similar to that of unaffected dentin.[17-19] If a poor technique is used, or contamination occurs during amalgam placement, it may elicit dentin staining, as can the presence of recurrent caries. Even in the late 19th and early 20th centuries, it was suggested that dentin staining could be caused by the deposition of metallic sulfides and the penetration of silver and mercury ions from the overlying amalgam.[19-25] Scholtanus and others[2] concluded that the discoloration of dentin beneath amalgam was an indicator of the presence of amalgam constituents, the effect of which is unknown in adhesive restor-

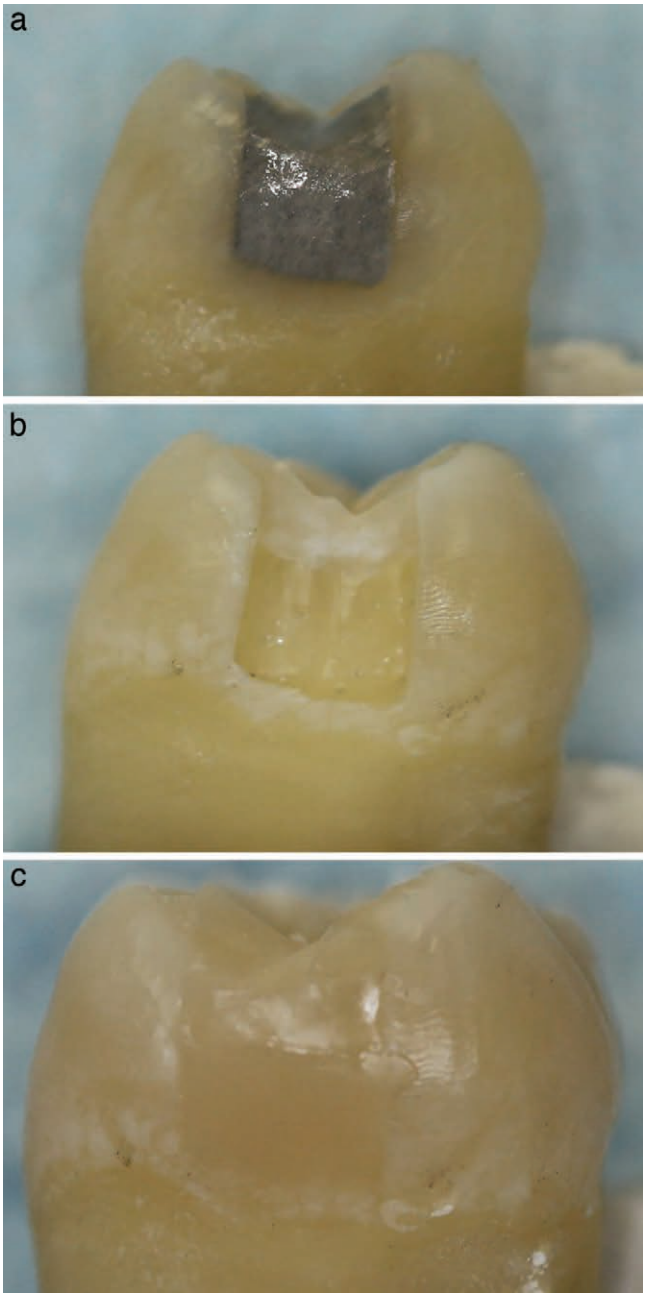


Figure 1a. *Restoration process a. amalgam restoration placement.*
Figure 1b. *Cavity walls after removal of the amalgam restoration.*
Figure 1c. *Composite resin placement.*

ative procedures. Harnirattisai and others[25] showed that bond strength to dark dentin after amalgam removal is lower than that to normal dentin. Data are lacking in the literature about microleakage of composite resins after amalgam removal.

Therefore, the purpose of this *in vitro* study was to evaluate the microleakage of composite restorations when bonded to a cavity previously restored with amalgam material compared with that of freshly cut dentin. The hypothesis was that there would be more microleakage in a composite restoration when bonded to a cavity previously restored with amalgam material compared with that of freshly cut dentin.

METHODS AND MATERIALS

Sample Selection and Preparation

Thirty intact, caries-free, extracted human molar teeth were collected from the general collection jar in the Oral Surgery Department at Tufts University School of Dental Medicine. Twenty teeth were used for the microleakage test and 10 for the SEM and energy dispersive x-ray (EDX) analysis.

The teeth were placed in autopolymerizing acrylic resin (Technovit, Heraeus Kulzer, Wehrheim, Germany) using a plastic mounting template (Ultradent, South Jordan, UT, USA) with the experimental surface of the teeth exposed. The teeth were stored in distilled water at room temperature at all times.

Fabrication of the Restoration

Standardized class II box preparations were prepared on the occluso-proximal surfaces of each tooth (4-mm bucco-lingual width and 2-mm mesio-distal depth) with the gingival cavosurface margin 1 mm above the cemento-enamel junction (CEJ) and the cavosurface margin a butt joint,[26] using #245 carbide burs (SS White, Lakewood, NJ, USA) in an air/water-cooled high-speed turbine. A new bur was used after every five preparations.[27]

A Tofflemire matrix band was placed, and the restoration procedure began with the application of two layers of varnish (Copalite, Cooley & Cooley LTD, Houston, TX, USA) according to the manufacturer's instructions. Each cavity was then restored using dental amalgam (Dispersalloy, Dentsply, Milford, DE, USA) (Figure 1a). All teeth were thermocycled for 10,000 thermal cycles between water baths at 5°C and 55°C with a 30-second dwell time. This procedure aged the material to simulate a year of clinical performance and to generate amalgam corrosion products.[28]

Next, the amalgam restoration was removed carefully using #245 carbide burs (SS White) in an air/water-cooled, high-speed turbine (Figure 1b). To prevent encroachment on the dentin, the final layer of amalgam was removed with an explorer.[29] The amalgam was removed from all the samples except five, which were used for the SEM and EDX analysis.

A cavity of the same dimensions was prepared on the other side of the tooth using #245 carbide burs (SS White) in an air/water-cooled high-speed tur-

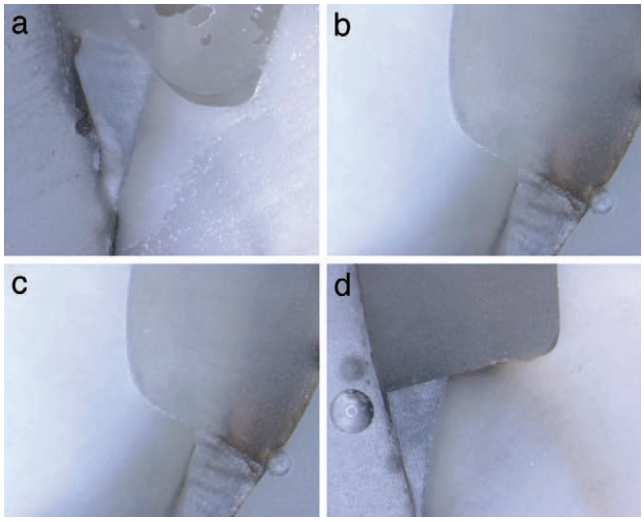


Figure 2a. *Microleakage at the proximal margin a. Score 0.*
Figure 2b. *Score 1.*
Figure 2c. *Score 2.*
Figure 2d. *Score 3.*

bine. A Tofflemire matrix band was placed to ensure that the light curing of the restoration occurred only from the occlusal side of the restoration. Both cavities were restored with composite material using a total-etch adhesive system.

The composite restoration began by etching the enamel with 35% phosphoric acid (Ultra-Etch, Ultradent) for 20 seconds and dentin for 15 seconds. The teeth were rinsed with water for 10 seconds and blot dried with cotton pellets to achieve a moist dentin surface and then were bonded with total-etch adhesive (ExciTE F DSC, Ivoclar Vivadent, Amherst, NY, USA) that was applied to the enamel and dentin surfaces, then agitated for 10 seconds and light cured according to the manufacturer's recommendations.

The teeth were restored with composite resin material (Filtek Supreme Ultra Universal, 3M ESPE, St. Paul, MN, USA) via the Liebenberg technique.[30] A blue light emitting diode (LED) was used at 800 mW/cm^2, followed by finishing and polishing (Figure 1c). The light source was monitored every five curing cycles using a radiometer (Demetron L.E.D, Kerr Corp., Orange, CA, USA) to ensure that the light intensity remained stable.[31] All the teeth were thermocycled for 5000 thermal cycles between water baths at 5°C and 55°C with a 30-second dwell time, which served to age the material to simulate clinical performance.

Microleakage Test Preparation

All of the samples were coated with two layers of nail polish (Vinyl shine nail polish, Rimmel London, London, UK), except for a 2.0-mm rim around the restoration to allow the leakage-tracing agent to contact the margins of the restoration. Thereafter, the teeth were immersed in a solution of 50 wt% ammoniacal silver nitrate (pH=9.5) (Fisher Scientific, Fair Lawn, NJ, USAQ) for 24 hours, followed by eight hours in a photo-developing solution (Eastman Kodak Co., Rochester, NY, USA).[32]

The specimens were washed under running water for one minute. The nail polish was removed carefully with a #15 scalpel, after which the specimen was embedded in epoxy material (Epoxicure resin, Buehler Ltd., Buehler, IL, USA). Each tooth was sectioned mesiodistally with a diamond saw, and the blocks were positioned in a precision cutting machine (1000 Isomet, Buehler Ltd.).

The specimens were analyzed with a stereomicroscope (Olympus America, Inc., Center Valley, PA, USA) at a magnification of 20×. All of the measurements were made in micrometers; two readings were taken for each cavity (occlusal and cervical = four readings per tooth).[33] The specimens were scored according to the following degree of dye penetration (Figures 2 and 3)—occlusal score: 0, no dye penetration; 1, dye penetration into enamel; 2, dye penetration beyond the dentinoenamel junction; 3, dye penetration into the pulpal wall; proximal score: 0, no dye penetration; 1, dye penetration into enamel; 2, dye penetration up to half extension of cervical wall; 3, dye penetration into more than half or complete extension of the cervical wall.[34]

The dye penetrations were also recorded in percentage measurements using the following formula[33]: leakage number = (distance evidenced for dye/overall distance for margin) × 100.

SEM and EDX

All of the samples were embedded in epoxy resin material (Epoxicure resin, Buehler Ltd.). Each sample was sectioned mesiodistally with a diamond saw (1000 Isomet, Buehler Ltd.). Each section was mounted on an Hitachi table (Aluminum mount, Electron Microscopy Science, Hatfield, PA, USA) with conductive adhesive (Graphite conductive adhesive, Electron Microscopy Science). The samples were coated with a 4.5-μm platinum layer using a coating machine (Sputter coater 208hr, Cressington, Watford, UK). Each section was analyzed with a scanning electron microscope (Hitachi S-48000, Hitachi, Tokyo, Japan) and EDX to analyze the metal penetration in the dentinal tubules below the interface between the restoration and the dentin, as

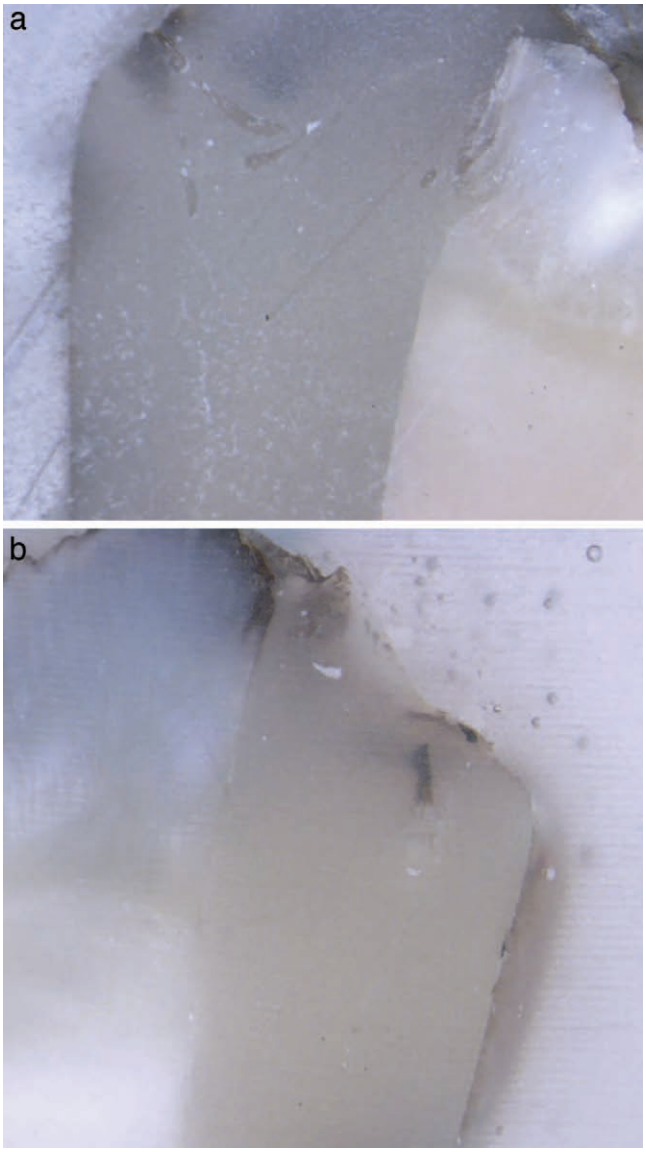


Figure 3a. *Microleakage at the occlusal margin a.Score 0.*
Figure 3b. *Score 1.*

well as at the interface. All analyses were conducted using EDAX Genesis software (EDAX Inc., Mahwah, NJ, USA) operated at 20 Kv.

Statistical Analysis

A power calculation was performed using nQuery Advisor (Version 7.0). Assuming an 84% chance for the group with nonpreviously restored teeth to have lower microleakage than the group with previously restored teeth,[35] a sample size of $n = 20$ teeth was adequate to obtain a type I error rate of 5% and a power greater than 99%.

For the 0-3 scale of microleakage at the cervical surface, counts and percentages were calculated, and statistical significance was assessed via the Wilcoxon signed-rank test. For the occlusal score, counts and percentages were calculated, and statistical significance was assessed via the McNemar test. For the percentage scale of microleakage, median and interquartile ranges were calculated, and statistical significance was assessed via the Wilcoxon signed-rank test, because the assumption of normally distributed data was violated; p-values less than 0.05 were considered statistically significant. (IBM SPSS Statistics 21, IBM Corp., Armonk, NY,USA) was used in the analyses.

Table 1: *Counts (Percentages) for 0-3 Microleakage Scale at Occlusal Margin*

Tested groups at occlusal margins	0	1	*p* Value
Previous amalgam	12 (60%)	8 (40%)	0.727
Fresh-cut dentin	10 (50%)	10 (50%)	

RESULTS

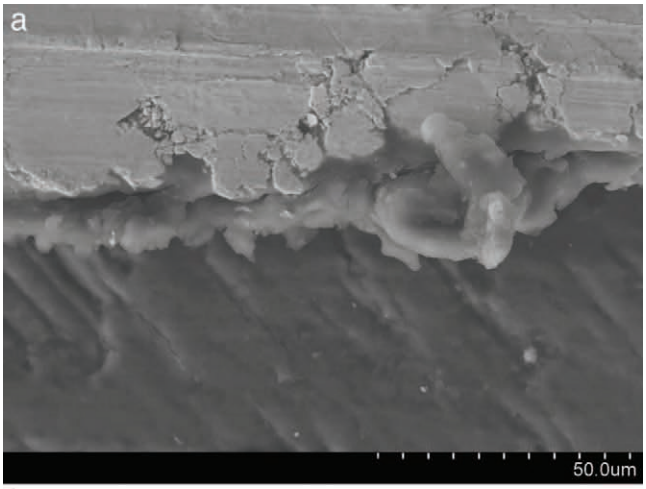
Microleakage Test

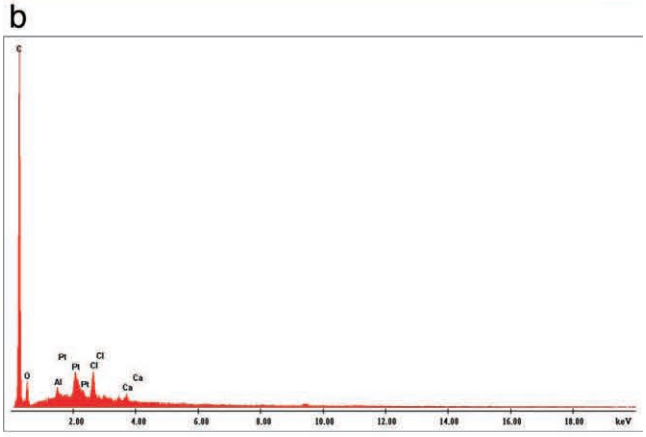
Dye penetration and consequently microleakage varied between the occlusal and proximal surfaces.

Occlusal Marginal Microleakage—The results of microleakage on a 0-3 scale are presented in Table 1. For the group of teeth that were previously restored with amalgam, 12 teeth (60%) had a score of 0, and eight teeth (40%) had a score of 1; for the fresh-cut dentin group, 10 teeth (50%) had a score of 0, and 10 teeth (50%) had a score of 1. The McNemar test revealed no significant difference in microleakage for the two groups at the occlusal surface (p=0.727).

Percentage measurement data are presented in Table 2. The median value for teeth that were previously restored with amalgam was 0%, whereas it was 13%; for the fresh-cut dentin group. The minimum percentage of microleakage for the amalgam group was 0%, with a maximum value of 16%. The minimum microleakage for the fresh-cut dentin was also 0%; the maximum value, however, was 33%. No significant difference was found between the groups (p=0.675).

Proximal Marginal Microleakage—The results of the microleakage on a 0-3 scale are presented in Table 3. For the teeth that were previously restored with amalgam, 18 teeth (90%) had a microleakage score of 2 or 3. For the fresh-cut dentin group, 13 teeth (65%) had a microleakage score of 2 or 3. The overall distribution of the microleakage scores did





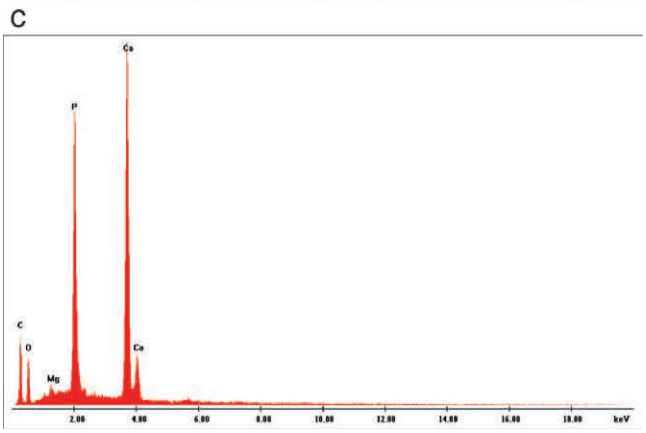


Figure 4a. *SEM of the dentin-amalgam interface showing the varnish layer.*
Figure 4b. *EDX analysis for varnish layer.*
Figure 4c. *EDX analysis for the dentin below the amalgam restoration.*

not differ significantly between the two groups (p=0.174).

Percentage measurement results are presented in Table 4. The median value for teeth that were previously restored with amalgam was 78% and for the fresh cut dentin was 67%. The minimum percentage of the microleakage for the amalgam group was 39%, with a maximum value of 100%. Although the maximum microleakage for the fresh-cut dentin group was also 100%, the minimum value was 0%. No significant difference was found between the groups (p=0.513). However, a statistically significant difference was found between the occlusal and proximal margins (p<0.001).

SEM and EDX

The results of the SEM and EDX are presented in graphs and elemental analysis charts.

Teeth With Amalgam Restorations (Figure 4a)—Several areas were analyzed (amalgam restoration, varnish layer, dentin surface, and the dentinal tubules beneath the restoration). The varnish layer revealed that chlorine was associated with the chloroform in the varnish composition (Figure 4b) and platinum metal from the coating. No metal was detected in the dentinal tubules below the amalgam restoration that was thermocycled for 10,000 or 15,000 cycles (Figure 4c).

Teeth That Were Previously Restored With Amalgam (Figure 5a)—No metal elements from the amalgam corrosion products were found in the dentinal tubules and the dentin surface beneath the composite restoration. However, silicon was detected and was found to be associated with the dentin adhesive (Figure 5b).

Teeth With Fresh-cut Dentin (Figure 6a)—Silicon was also detected to be associated with the dentin adhesive. However, there were differences between the amount of silicon in the hybrid layer and the dentin beneath the restoration (Figure 6b).

DISCUSSION

In this study, dye penetration testing was chosen because it is the most widely used method of evaluation and is the gold standard. Dye penetration also represents the most reliable quantitative mea-

Table 2: *Descriptive Statistics for the Percentage Microleakage Score at the Occlusal Margin*

Tested groups at occlusal margins	*N*	Median	Interquartile range	Minimum	Maximum	*p* Value
Previous amalgam	20	0	8	0	16	0.675
Fresh-cut dentin	20	13	7	0	33	

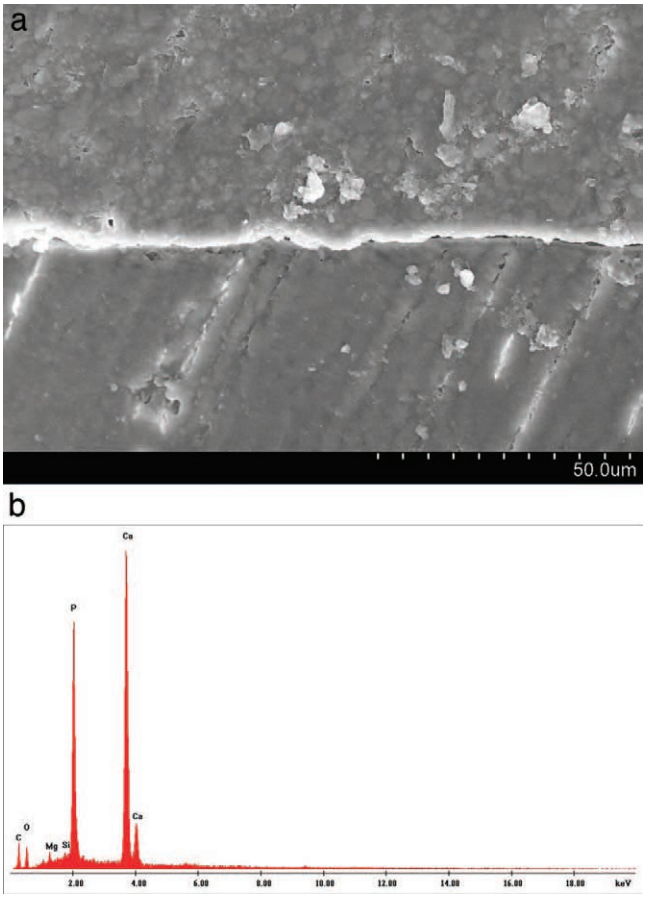


Figure 5a. *SEM of the dentin-composite interface that was previously restored with amalgam.*
Figure 5b. *EDX analysis for the dentin below composite restoration.*

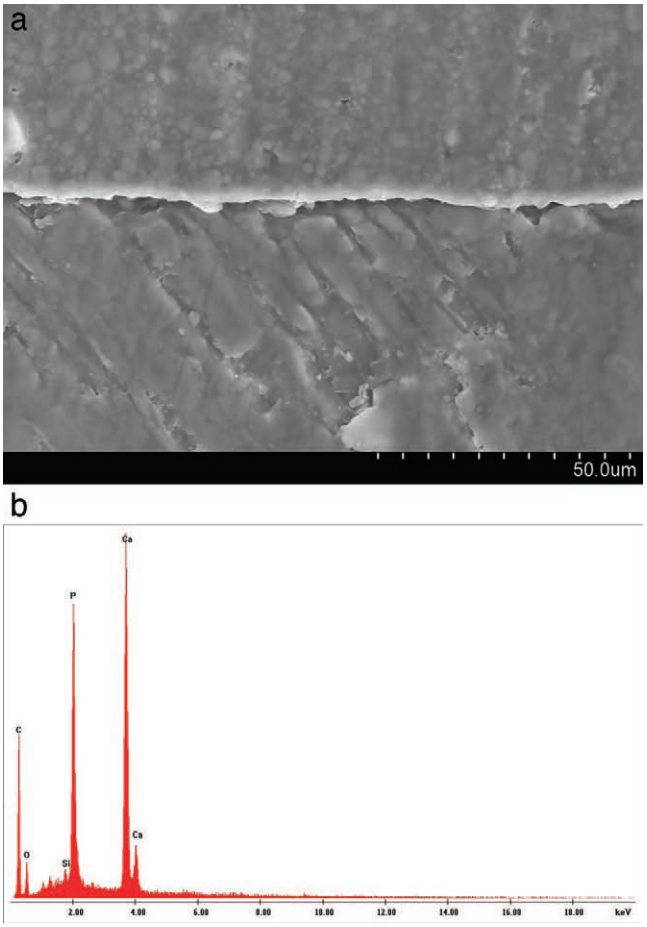


Figure 6a. *SEM of the dentin-composite interface for fresh-cut dentin.*
Figure 6b. *EDX analysis for the dentin below the composite restoration.*

surement of microleakage. One of the goals of an ideal restoration is to prevent microleakage, as this is an important aspect of the longevity of restorations. Moreover, it represents the passage of bacteria at the tooth restoration interface, which may cause recurrent caries or pulpal irritation and subsequent pulpal inflammation.[36]

Two measurements were used to evaluate the dye penetration at the proximal and occlusal margins to confirm whether or not different measuring techniques might affect the results. Hypothesis tests based on the two measurements led to the same conclusion. All teeth previously restored with amalgam restorations had 90% microleakage at the proximal margin with scores of 2 or 3. In contrast, the other group showed 65% microleakage with the same scores.

This may be due to the effect of the cavity preparation and application of a cavity varnish layer. The smear layer and subsequent contaminants could block the dentinal tubules from forming a smear plug. Contaminated dentin could be exposed to a variety of ions and molecules originating from amalgam and oral fluids. Further, differences in the coefficient of thermal expansion between amalgam and tooth structure can result in intermittent opening and closing of the gap, thus

Table 3: *Counts (Percentages) at the Proximal Margin*

Tested groups at proximal margins	**0**	**1**	**2**	**3**	***p* Value**
Previous amalgam	0 (0%)	2 (10%)	12 (60%)	6 (30%)	0.174
Fresh cut dentin	2 (10%)	5 (25%)	8 (40%)	5 (25%)	

Table 4: *Descriptive Statistics for the Percentage Microleakage Score at the Proximal Margin*

Tested groups at proximal margins	*N*	Median	Interqartile range	Minimum	Maximum	*p* Value
Previous amalgam	20	78	38	39	100	0.513
Fresh-cut dentin	20	67	52	0	100	

creating inward and outward transport of fluids along the amalgam-tooth interface. Dentin is exposed to saliva and products of bacterial metabolism more readily,[2] and these factors elucidate the difference between the dentin of teeth that were previously restored with amalgam compared to fresh-cut dentin.

The findings of this study contrasted with those of Ghavamnasiri and others,[29] who found a difference in microleakage between teeth that were previously restored with amalgam vs with freshly cut dentin. This difference may be related to varied composition or solutions, as Ghavamnasiri and others used chloroform-free varnish. Other differences that may have had an influence on the results include dye tracer and storage methods. According to Heintze and others,[37] different tracers produce different results, especially at the dentin margin.

The EDX analysis in this study revealed the presence of calcium and phosphorus, elements that are correlated with the composition of dentin.[38] The findings of this study are consistent with those of Wei and Ingram, Kurosaki and Fusayama, and Halse and others, who concluded that metal did not penetrate into nondiscolored dentin. They found that penetration of metals, such as Zn and Sn, occurred exclusively in the dark, discolored dentin.[39-41] The review of the literature by Scholtanus and others[2] confirmed that penetration of metals from amalgam was observed only in discolored and demineralized dentin.[2,25,40-43]

However, these results conflict with that of the research of Ghavamnasiri and others,[29] as tin, silver, mercury, and copper were found in the dentin adjacent to the composite restoration after the amalgam replacement. Moreover, high copper amalgam was used, and it has been reported that no mercury is released by the corrosion process. Corrosion of high copper amalgam with in vitro experiments revealed Ca-Sn-P-Cl complexes and crystalline products containing Sn at the amalgam-tooth interface. No Cu was found as Cu complexes were assumed to be leached out into the liquid environment.[2,29]

The findings of this study conflict with those of the study of Grossman and Matejka,[38] as several metal elements were found in the dentin and enamel after the placement of the amalgam restorations. However, a conventional amalgam was used, and the samples were stored in 1% NaCl solution for one year, which may have affected the corrosion process or products. Moreover, Soremark and others[44] also found an increase in the concentrations of mercury and silver in the dentin and enamel after the amalgam restoration, as well as a moderate increase in tin and zinc. In this particular study, the researchers did not specify the amalgam type, which has a significant effect on the corrosion products.

The EDX analysis of the dentin below the composite restorations also revealed differing amounts of silicon, which related to the application of the adhesive. This finding contrasts with that of Harnirattisai and others,[25] who used Single Bond (3M ESPE) and Clearfil SE Bond (Kuraray, New York, NY, USA) and did not find elements related to the adhesive in the adjacent dentin. Our findings are consistent with those of Ghavamnasiri and others,[29] who reported the presence of large amounts of tin, barium, and silicon; the concentrations of both tin and barium were related to the opaque metal included in the adhesive.

Although there was no statistically significant difference between the two groups, and metal did not penetrate the nondiscolored dentin, the teeth previously restored with amalgam restorations showed more microleakage (39% to 100%) compared with the freshly cut dentin that had (0% to 100%). These findings suggest that cavity preparation should be extended beyond the removal of an amalgam restoration. Ghavamnasiri and others[29] also recommended the removal of approximately 0.5 mm of nondiscolored dentin to improve the gingival microleakage and achieve the same level as that obtained with the fresh dentin composite restoration.

Because several different steps were involved in our study, including storage, thermocycling, manipulation, and dye penetration, among others, the different results simply may be caused by variation error in any of the aforementioned procedures.[45] Adequate research and data on the microleakage of composite resin restorations after replacing high

copper amalgam with nondiscolored dentin is lacking. Harnirattisai and others[25] concluded that the bond strength of discolored dentin after amalgam removal was less than that of normal dentin. However, they did not test microleakage in their study.

The limitations of this study include but are not limited to 1) insufficient *in vitro* data for comparison, 2) artificial aging (thermocycling) without salivary intervention, and 3) dye penetration in a two-dimensional model.

Further research is recommended in which a three-dimensional analysis and thermomechanical loading are used, as these are more analogous to the oral environment. Although clinical trials remain ideal for the evaluation of dental restorations, the results of this study provide scientific evidence for composite resin microleakage after amalgam restoration replacement.

Within the limitations of the study, the following conclusions can be drawn: no significant difference was found between the microleakage of nondiscolored dentin in teeth that were previously restored with amalgam compared with freshly cut dentin, and marginal microleakage in the proximal surface was higher than that in the occlusal surface.

Regulatory Statement

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

Conflict of Interest

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

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Mechanical Properties and Sliding-impact Wear Resistance of Self-adhesive Resin Cements

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Clinical Relevance

Mechanical properties and wear resistance are important parameters for the selection of a resin luting cement. For wear resistance, the self-adhesive resin cements generally show significantly lower values than the conventional resin cements.

SUMMARY

The present study determined the mechanical properties and impact-sliding wear characteristics of self-adhesive resin cements. Five self-adhesive resin cements were used: G-CEM LinkAce, BeautiCem SA, Maxcem Elite, Clearfil SA Automix, and RelyX Unicem 2. Clearfil Esthetic Cement was employed as a control material. Six specimens for each resin cement were used to determine flexural strength, elastic modulus, and resilience according to ISO specification #4049. Ten specimens for each resin cement were used to determine the wear characteristics using an impact-sliding wear testing apparatus. Wear was generated using a stainless-steel ball bearing mounted inside a collet assembly. The maximum facet depth and volume loss were determined using a noncontact profilometer in combination with confocal laser scanning microscopy. Data were evaluated using analysis of variance followed by the Tukey honestly significantly different test (α=0.05). The flexural strength of the resin cements ranged from 68.4 to 144.2 MPa; the elastic modulus ranged from 4.4 to 10.6 GPa; and the resilience ranged from 4.5 to 12.0 MJ/m^3. The results for the maximum facet depth ranged from 25.2 to 235.9 μm, and volume loss ranged from 0.0107 to 0.5258 mm^3. The flexural properties and wear resistance were found to vary depending upon the self-adhesive resin

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cement tested. The self-adhesive cements tended to have lower mechanical properties than the conventional resin cement. All self-adhesive resin cements, apart from G-CEM LinkAce, demonstrated significantly poorer wear resistance than did the conventional resin cement.

INTRODUCTION

Self-adhesive resin cements utilized as luting agents are defined as cements based on filled polymers that adhere to the tooth structure in the absence of tooth surface pretreatment.[1,2] Therefore, self-adhesive resin cements are considered easier to apply compared with conventional resin cements.[3] The application procedure is simple, and no postoperative sensitivity is expected because the smear layer is not removed during the cementation process. These cements contain acidic functional monomers that demineralize the tooth structure and promote infiltration of the resin components into the etched tooth substrate.[1,2] In addition, the setting reactions of most self-adhesive resin cements use a dual curing process based on the incorporation of photo-initiators along with redox initiators. However, in contrast to their simplified application, the compositions of self-adhesive resin cements are complicated. Moreover, incompatibility between acidic functional monomers and other resinous components can have adverse effects on the mechanical properties of self-adhesive resin cements. Nevertheless, previous studies[4,5] have demonstrated that self-adhesive resin cements are mechanically stronger than zinc phosphate, zinc polycarboxylate, glass ionomer, and resin-modified glass ionomer cements when comparing their flexural and compressive strengths. In addition, it has been established that self-adhesive resin cements possess similar flexural strengths to those of conventional resin cements.[6-8]

From the standpoint of clinical conditions, one of the challenges for luting cements is marginal integrity. To select suitable luting cements for specific clinical conditions, it is important to evaluate the wear performance characteristics of the cement to achieve long-lasting restorations. Marginal gap formation may lead to a variety of concerns, including marginal staining, secondary caries, bonding failure, restoration fracture, and postoperative sensitivity.[9] In addition, dental clinicians require more information related to the clinical relevance of the mechanical properties of more recently developed self-adhesive resin cements, including detail about their wear resistance.

Several studies[10,12] have evaluated marginal gaps in an effort to assess the potential for cement loss at the margins of restorations and to yield more information concerning the expected quality of restorations. However, little information exists concerning the wear resistance properties of recently developed self-adhesive resin cements. The purpose of the present study was to assess the flexural properties and wear properties of self-adhesive resin cements and to compare these properties with those of a conventional resin cement. The null hypothesis to be tested was that there are no significant differences in wear and mechanical properties between the conventional resin cement and the self-adhesive resin cements.

METHODS AND MATERIALS

Materials Used

Five self-adhesive resin cements were tested: G-CEM LinkAce (GL; GC Corp., Tokyo, Japan); BeautiCem SA Auto-mixing (BC; Shofu Inc, Kyoto, Japan); Maxcem Elite (ME; Kerr Corp, Orange, CA, USA); Clearfil SA Luting Automix (SA; Kuraray Noritake Dental Inc, Tokyo, Japan); and RelyX Unicem 2 (RU; 3M ESPE Dental Products, St Paul, MN, USA). A conventional resin cement, Clearfil Esthetic Cement (EC; Kuraray Noritake Dental Inc), was employed as a control material. The test materials and their components are listed in Table 1. An Optilux 501 visible–light-curing unit (sds Kerr, Danbury, CT, USA) was employed, and the light irradiance (average 800 mW/cm^2) of the curing unit was checked using a dental radiometer (Model 100, Kerr).

Inorganic Filler Content

The inorganic filler content of the materials was measured using thermogravimetry/differential thermal analysis employing a 6300 thermogravimeter (Seiko Instruments, Tokyo, Japan). For each resin cement tested, a mixed cement paste sample (approximately 50 mg) was heated in the thermogravimeter from 25°C to 800°C at a heating rate of 10°C/min until the organic components were completely consumed. The weight of the residual cement paste was measured and the inorganic filler content (wt%) was calculated. Three measurements were conducted to obtain an average inorganic filler content (wt%).

Coefficient of Linear Thermal Expansion

The coefficients of linear thermal expansion of the test materials were measured using a thermome-

Table 1: *Primary Characteristics of the Six Cement Materials Studied*

Self-adhesive Resin Cements	Shade	Manufacturer	Main Components[a]	Code
G-CEM LinkAce Lot No. 1402271	A2	GC Corp, Tokyo, Japan	UDMA, dimethacrylate, phosphonate monomer, *y*-methacryloxypropyltrimethoxysilane, α, α-dimethylbenzyl hydroperoxide, fluoro alumino silicate glass, silicon dioxide, initiator, inhibitor, pigment	GL
BeautiCem SA Lot No. 081346	Ivory	Shofu Inc, Kyoto, Japan	UDMA, HEMA, carboxylic acid monomer, phosphonate monomer, fluoro alumino silicate glass, zirconium silicate filler (amorphous), polymerization initiator, others	BC
Maxcem Elite Lot No. 4947576	Brown	Kerr Corp, Orange, CA, USA	Bis-GMA, UDMA, GPDM, glyceroldimethacrylate, mono-, di-, and multi-methacrylate co-monomers, CQ, barium alumino borosilicate glass, fluoro alumino silicate glass, stabilizer, others	ME
Clearfil SA Automix Lot No. 6L0011	A2	Kuraray Noritake Dental Inc, Tokyo, Japan	Bis-GMA, TEGDMA, MDP, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, silanated barium glass filler, silanated colloidal silica, surface-treated sodium fluoride, CQ, initiator, benzoyl peroxide, accelerators, pigments	SA
RelyX Unicem 2 Lot No. 6L0011	A2	3M ESPE Dental Products, St Paul, MN, USA	Propanediyl dimethacrylate and phosphorus oxide, substitute dimethacrylate, TEDGMA, 2-propenoic acid, 2-methyl-, 1,1′-[1-(hydroxymethyl)-1,2-ethanediyl]ester, silane-treated glass powder, silane-treated silica, sodium persulfate, glass powder, *tert*-butyl peroxy-3,5,5-trimethylhexanoate, copper (II) acetate monohydrate, 1,12-dodecane dimethacrylate, 1-benzyl-5-phenyl-barbic-acid, calcium salt, sodium *p*-toluenesulfinate, calcium hydroxide, methacrylated aliphatic amine, titanium dioxide	RU
Esthetic Cement Lot No. 0037AA	A2	Kuraray Noritake Dental Inc, Tokyo, Japan	Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated barium glass filler, silanated silica filler, colloidal silica, benzoyl peroxide, accelerator, CQ, initiators, pigments, others	EC

Abbreviations: Bis-GMA, 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane; CQ, DL-camphorquinone; GPDM, glyceroldimethacrylate dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethyleneglycol dimethacrylate; UDMA, urethane dimethacrylate.
Indicate no statistically significant differences among its members (Tukey honestly significantly different [HSD] test, $p > 0.05$).
[a] *According to each manufacturer's Material Safety Data Sheet.*

chanical analyzer (TMA/SS 6300, Seiko Instruments). For each resin cement tested, the mixed paste was compacted into a stainless-steel split mold of dimensions 25 mm × 2 mm × 2 mm, which was positioned on a glass slide. For resin cement curing within the mold, the central 5-mm section, followed by the adjacent sections, was irradiated for 30 seconds, and, finally, each end was irradiated in turn for the same period. The specimens were stored under dark conditions at 25°C for 24 hours and subsequently fixed in a vice and cut in half (for a length of 12.5 mm) with a diamond saw before the measurements were conducted. Three specimens for each material were prepared and separately tested in the thermomechanical analyzer at a heating rate of 2°C/min from 25°C to 130°C. The average coefficient of linear thermal expansion ($\times 10^{-6}$/°C), over a temperature range 30°C to 80°C, was thereby obtained.

Flexural Strength and Elastic Modulus Measurement

The flexural properties were tested according to ISO specification #4049. The test samples were obtained following an equivalent procedure to that described above for evaluation of the coefficients of linear thermal expansion, except that they were not cut in half. After the removal of the hardened specimen from the mold, all six surfaces were wet ground with #1200 silicon carbide (SiC) papers (Fuji Star Type DDC, Sankyo Rikagaku Co, Saitama, Japan). The specimens were then stored for 24 hours in distilled water at 37°C prior to testing. Six specimens for each of the six resin cements were

subjected to a three-point bending flexural strength test using a universal testing instrument (Type 5500R, Instron Corp, Canton, MA, USA) with a span length of 20.0 mm at a cross-head speed of 1.0 mm/min until the specimen fractured. The specimens were kept moist during testing. The peak breaking stress, modulus of elasticity, and resilience were determined from the stress-strain curve of each sample using the Bluehill Ver 2.5 computer software (Instron Corp) linked to the testing instrument.

Impact-sliding Wear Simulation

Ten specimens for each of the six resin cements were tested to determine the wear behavior using the impact-sliding wear testing apparatus (K655-05, Tokyo Giken Inc, Tokyo, Japan). For each specimen, the mixed resin cement paste was placed into a cylindrical Teflon mold (6 mm in diameter, 2 mm in height). The cement paste was cured for 30 seconds and stored under dark conditions for 24 hours in distilled water at 37°C. The flat surfaces of each specimen were polished to 4000 grit using a graded sequence of SiC papers. Specimens were attached with a small amount of model-repair glue (Zapit, Dental Ventures of America Inc, Corona, CA, USA) to the centers of custom fixtures fabricated from a cold-cure acrylic resin (Tray Resin II, Shofu Inc).

The antagonist for the impact-sliding wear simulation was a stainless-steel ball bearing of radius $r = 2.387$ mm that was mounted inside a collet assembly. The simulator incorporated a plastic water bath that provided water at a temperature of 37°C constantly, and four of the custom wear fixtures were mounted inside the bath. During the course of the wear simulation test, the antagonists directly impacted the specimens from above with a maximum force of 50 N at a rate of 0.5 Hz, and the antagonists subsequently slid horizontally for a distance of 2 mm. Each specimen was subjected to 50,000 cycles of the impact-sliding motion.

Wear Measurements

Following the wear simulation test, the specimens were ultrasonically cleaned in distilled water for three minutes and then profiled using a confocal laser scanning microscope (VK-9710, Keyence, Osaka, Japan) with built-in analysis software (VK analyzer, Keyence). The maximum depth (μm) and volume loss (mm^3) of the wear facets were thereby determined.

Scanning Electron Microscopy Observations

The surfaces of the cured resin cement samples were polished to a high gloss with abrasive discs (Fuji Star Type DDC, Sankyo Rikagaku Co) followed by a series of diamond pastes down to a 0.25-μm particle size (DP-Paste, Struers, Ballerup, Denmark). The polished surfaces were then subjected to unfiltered argon-ion beam etching (IIS-200ER, Elionix, Tokyo, Japan) for 40 seconds perpendicular to the polished surface at an accelerating voltage of 1.0 kV and ion current density of 0.4 mA/cm^2. The surfaces were then coated in a vacuum evaporator (Quick Coater Type SC-701; Sanyu Denshi Inc, Tokyo, Japan) with a thin film of gold. Examinations of the surfaces were conducted by scanning electron microscopy (SEM) using an Elionix FE-8000 instrument (Elionix, Tokyo, Japan) with an operating voltage of 10 kV and a magnification of 5000×.

SEM examinations were conducted on the wear facets of the six resin cement samples after impact-sliding wear simulation. Representative samples for each resin cement were sputter-coated with gold. Examinations of the coated surfaces were conducted using the SEM with an operating voltage of 10 kV and magnifications of 40× and 2500×.

Statistical Analysis

The data for each material were subjected to analysis of variance followed by the Tukey honestly significantly difference (HSD) test at a significance level of 0.05. The statistical analysis was conducted using the Sigma Plot software system (Ver. 11.0; SPSS Inc, Chicago, IL, USA).

RESULTS

Inorganic Filler Content and Coefficient of Thermal Expansion

The average inorganic filler contents and coefficients of thermal expansion are listed in Table 2. The average inorganic filler contents of the resin cements ranged from 55.3 to 67.9 wt%. The highest inorganic filler content was determined for EC, whereas GL showed the lowest value of all the test materials, and these differences were statistically significant. The average coefficients of thermal expansion of the resin cements ranged from 37.7 to 51.6 $\times 10^{-6}$/°C. A significantly low value was exhibited by RU, whereas ME showed a significantly higher value than all other tested resin cements.

Table 2: *Inorganic Filler Contents and Thermal Expansion Coefficients of the Cement Materials Studied*

Code	Inorganic Filler Content, wt%[a]	Tukey Group[b]	Thermal Expansion Coefficient, $\times 10^{-6}$/°C (from 30°C to 80°C)[a]	Tukey Group[b]
GL	55.3 (0.2)	F	44.7 (1.6)	B
BC	59.6 (0.2)	E	42.8 (1.5)	B
ME	66.9 (0.2)	B	51.6 (0.8)	A
SA	65.8 (0.2)	C	45.3 (0.9)	B
RU	63.6 (0.2)	D	37.7 (0.2)	C
EC	67.9 (0.3)	A	41.8 (2.0)	B

Abbreviations: BC, BeautiCem SA; EC, Esthetic Cement; GL, G-CEM LinkAce G-CEM LinkAce; ME, Maxcem Elite; RU, RelyX Unicem 2; SA, Clearfil SA Automix.
[a] Mean (standard deviation), n = 6.
[b] Equivalent group letters indicate no statistically significant differences among its members (Tukey honestly significantly different [HSD] test, p>0.05).

Flexural Properties

The results of flexural property testing are listed in Table 3. The average flexural strength of the resin cements ranged from 68.4 to 144.2 MPa, and the average elastic modulus ranged from 4.4 to 10.6 GPa. Tukey HSD test indicated that BC exhibited the highest flexural strength of all test resin cements, and no significant difference was obtained when BC was compared to the control material EC. Regarding the elastic modulus, EC demonstrated the highest value. Self-adhesive resin cements BC, GL, and RU exhibited higher values compared to all other self-adhesive resin cements, and no significant differences were obtained among them. The average resilience ranged from 4.5 to 12.0 MJ/m^3. The control material EC exhibited the highest resilience.

Impact-sliding Wear

The average wear values (maximum facet depth and volume loss) for the six resin cements are summarized in Table 4. The rank orders of all six resin cements were similar with regard to both facet wear depth and volumetric loss. Cements EC and GL demonstrated significantly lower maximum facet depth and volume loss when compared to all other resin cements. In contrast, ME exhibited the highest maximum facet depth and volume loss when compared to all other resin cements, and all differences were statistically significant.

Table 4: *Maximum Facet Depth and Volume Loss of the Cement Materials Studied*

Code	Maximum Facet Depth, μm[a]	Tukey Group[b]	Volume Loss, mm^3[a]	Tukey Group[b]
GL	28.6 (1.5)	E	0.0110 (0.003)	D
BC	122.3 (31.7)	D	0.1712 (0.059)	C
ME	239.5 (13.9)	A	0.5258 (0.051)	A
SA	187.5 (30.8)	C	0.3075 (0.103)	B
RU	197.8 (44.9)	B	0.2060 (0.046)	C
EC	25.2 (10.2)	E	0.0107 (0.005)	D

Abbreviations: BC, BeautiCem SA; EC, Esthetic Cement; GL, G-CEM LinkAce G-CEM LinkAce; ME, Maxcem Elite; RU, RelyX Unicem 2; SA, Clearfil SA Automix.
[a] Mean (standard deviation), n = 6.
[b] Equivalent group letters indicate no statistically significant differences among its members (Tukey honestly significantly different [HSD] test, p>0.05).

SEM Observations

SEM examinations of the six resin cements after argon-ion etching are presented in Figure 1a-f. Differences in filler particle size, shape, and distribution are clearly observable. For GL, RU, and ME, 0.5-5-μm irregularly shaped glass filler particles are observed, as shown in Figure 1a, c, and e, respectively. For BC, 0.2-5 μm–sized spherical silica filler particles and 0.2-1 μm irregular glass filler particles are observed in Figure 1b. SA and EC include relatively large irregular glass filler particles mea-

Table 3: *Flexural Strength, Elastic Modulus, and Resilience of the Cement Materials Studied*

Code	Flexural Strength, MPa[a]	Tukey Group[b]	Flexural Modulus, GPa[a]	Tukey Group[b]	Resilience, MJ/mm^3[a]	Tukey Group[b]
GL	121.4 (7.9)	B	7.2 (0.4)	B	9.5 (2.1)	B
BC	144.2 (12.2)	A	7.7 (0.5)	B	8.5 (2.0)	B,C
ME	68.4 (3.9)	D	4.4 (0.3)	D	4.5 (0.5)	D
SA	86.6 (8.5)	C	5.3 (0.5)	C	6.3 (1.5)	C,D
RU	99.7 (8.6)	C	7.2 (0.4)	B	6.0 (0.9)	C,D
EC	138.5 (3.8)	A	10.6 (0.8)	A	12.0 (2.0)	A

Abbreviations: BC, BeautiCem SA; EC, Esthetic Cement; GL, G-CEM LinkAce G-CEM LinkAce; ME, Maxcem Elite; RU, RelyX Unicem 2; SA, Clearfil SA Automix.
[a] Mean (standard deviation), n = 6.
[b] Equivalent group letters indicate no statistically significant differences among its members (Tukey honestly significantly different [HSD] test, p>0.05).

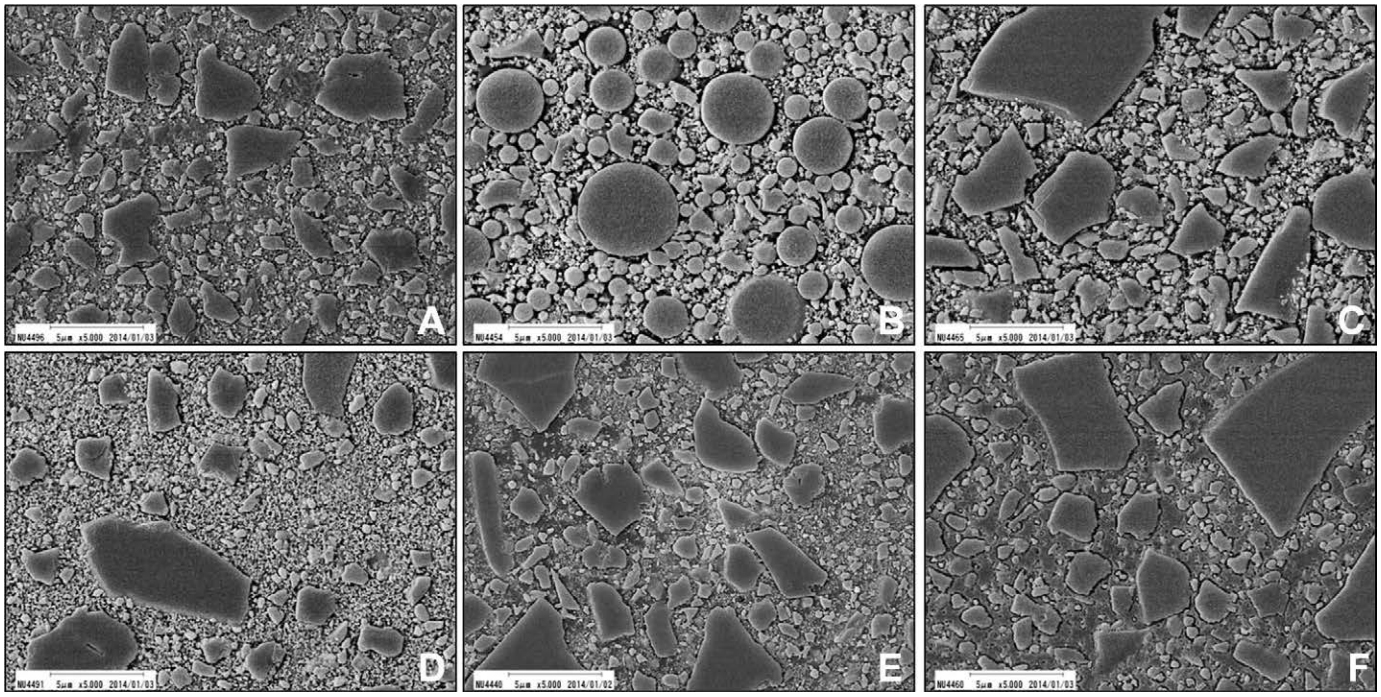

Figure 1. *SEM micrographs at a 5000× magnification of the argon-ion etched surfaces of the six cement materials studied, as follows. (A) G-CEM LinkAce argon-ion etched surface at 5000×. (B) BeautiCem SA argon-ion etched surface at 5000×. (C) Maxcem Elite argon-ion etched surface at 5000×. (D) Clearfil SA Automix argon-ion etched surface at 5000×. (E) RelyX Unicem 2 argon-ion etched surface at 5000×. (F) Esthetic Cement argon-ion etched surface at 5000×.*

suring approximately 0.2-10 μm in size, as shown in Figure 1d and f.

Representative SEM images of wear facets after impact-sliding wear testing are presented in Figure 2a-h. For GL and EC, the SEM examinations given in Figure 2a and g, respectively, reveal relatively flat and smooth surfaces, although some fine cracks are observed on the worn surfaces, as shown in Figures 2b and h for GL and EC, respectively. However, all other self-adhesive resin cements exhibited rougher

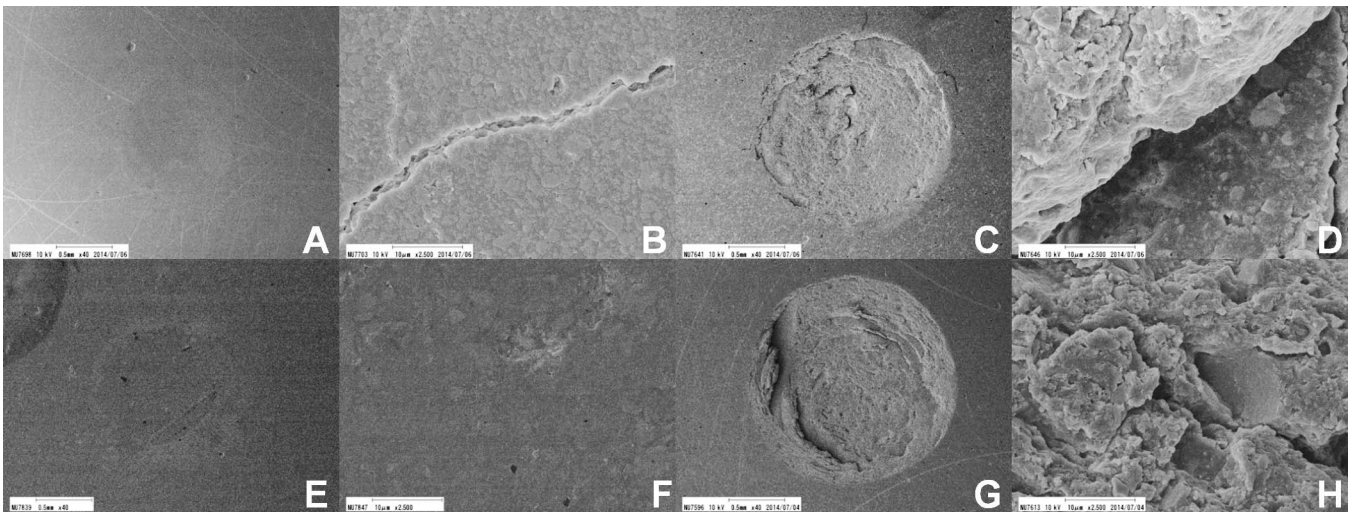

Figure 2. *SEM micrographs at 40× and 2500× magnifications of the surfaces of representative samples of the six cement materials subjected to impact-sliding wear testing. (A) G-CEM LinkAce Impact-sliding wear facet at 40×. (B) G-CEM LinkAce Impact-sliding wear near center of facet at 2500×. (C) Clearfil SA Automix Impact-sliding wear facet 40×. (D) Clearfil SA Automix Impact-sliding wear near center of facet at 2500×. (E) RelyX Unicem 2 Impact-sliding wear facet 40×. (F) RelyX Unicem 2 Impact-sliding wear near center of facet at 2500×. (G) Esthetic Cement Impact-sliding wear facet 40×. (H) Esthetic Cement Impact-sliding wear near center of facet at 2500×.*

and deeper facets than those of either GL or the control material EC. Similar wear patterns are observed for the more highly worn self-adhesive resin cement samples, with some plucking of filler particles from the surface and obvious cracks, as shown in Figure 2c-f.

DISCUSSION

Improvements in the resin matrix and filler components of direct composite restorations have resulted in markedly improved materials for applications in the posterior region.[13,14] However, dental professionals remain concerned about the mechanical properties and wear resistance of these materials for application to larger posterior lesions. Indirect restorations have been selected, rather than direct restorations, as the standard procedure for the posterior region because of their durable properties, ease of fabrication in a precise anatomical form, and adaptation to adjacent teeth.[15] At present, metal alloys, resin composites, and ceramics are used as indirect restorative materials. However, to achieve durable bonds between an indirect restoration and the surrounding tooth structure, each of these materials requires several pretreatment steps for luting.

Therefore, simplified self-adhesive resin cements for luting of restoratives have been developed that can reduce the clinical steps required for the luting procedure, mitigate technique sensitivity, and diminish postoperative sensitivity.[16,17] In addition, to avoid an insufficient degree of conversion when light delivery from a curing unit is inhibited as a result of obstructions by overlying indirect restorations, self-adhesive resin cements have been developed that incorporate a dual-polymerization system. This type of cement contains inorganic fillers, matrix resins, initiator systems, and acidic functional monomers. However, a major concern has been expressed regarding a reduced degree of conversion due to the presence of acidic functional monomers because of the risk of lower pH[18,19] and interference with the reaction between the tertiary amine and camphorquinone.[20-22] Therefore, to predict *in vivo* performance of self-adhesive resin cements, it is important to determine their endurance characteristics by evaluating their mechanical properties and wear resistance.

The results for inorganic filler content and thermal expansion of the resin cements appeared to be material dependent. Regarding the filler content, it is considered that increasing the amount of inorganic filler content plays an important role in enhancing the mechanical properties, reducing the polymerization shrinkage, and increasing the wear resistance. Although a high filler content is desirable for resin-based materials, it is difficult to include a large amount of filler because of the need to maintain an appropriate viscosity for luting and to ensure a suitable film thickness.

Thermal expansion is an important property of luting cements when considering the longevity of restorations. Temperature cycling may induce dimensional changes in the materials and rupture of the bond interface not only between the luting cement and the tooth structure, but also between the luting cement and the restoration as a result of differences in the thermal expansion properties of the individual materials. Because the thermal expansion of the restorative material and luting cements usually does not match that of the tooth structure, a differential expansion occurs that may result in leakage of oral fluids into the interface between the restoration and the tooth. From the results of this study, thermal expansion of the six resin cements ranged from 37.7 to 51.6 $\times 10^{-6}$ m/°C. In addition, in spite of the higher inorganic filler content, ME exhibited a significantly higher average thermal expansion coefficient than all other resin cements. A higher inorganic filler content has been considered to reduce thermal expansion, but the results obtained in this study suggested that other factors, such as the type of matrix resin and size of the filler, might have a significant influence on the thermal properties. Considering the long-term durability of indirect restorations, further improvement of thermal properties is needed in the resin cements.

Fracture-related material properties, such as fracture resistance, elasticity, and the marginal degradation of materials under stress, have typically been evaluated by determining material parameters such as flexural strength, flexural modulus, and fracture toughness.[23,24] Although flexural strength under constant loading may not reflect intraoral conditions, these values are helpful in comparing materials under controlled conditions.[25] From the results of flexural testing, the control material EC tended to have a higher flexural strength than the self-adhesive cements considered. However, BS and GL demonstrated a flexural strength similar to that of EC. A previous study revealed a correlation between the filler content, filler size, and the distribution of filler particles in resin-based materials and their material properties.[26,27] However, analysis of those results in this study showed no significant correlation, so other factors appear to be

more important. In addition, it is speculated that the functional monomers contained in self-adhesive cements might affect the degree of conversion of the cement itself.

It has been suggested that the desirable modulus of elasticity for a luting cement lies between those of the restorations and mineralized tooth structure, which contributes to a reduction of interfacial stress concentrations. Furthermore, resistance to plastic deformation may increase the resistance to marginal gap formation and cement fracture, and therefore a high proportional limit and resilience are preferred.[28] For resilience, the self-adhesive cements tended to exhibit lower values than that of the control material EC. Thus, the lower elastic modulus and resilience of self-adhesive cements may remain a disadvantage in preventing deformation and fracture of brittle restorations, such as all-ceramic and machinery-milled restorations.

Many types of filler systems, monomer systems, and coupling agents have been developed to improve the mechanical properties and wear resistance of resin composites. Resin luting cements, including self-adhesive resin cements, have been developed using resin composite technology. Thus, these types of materials might have similar characteristics to those of resin composites, and the wear resistance of the cement itself should be evaluated. Wear progression of luting cements at the margin may lead to gaps between a restoration and the surrounding tooth structure and may create a vulnerable region that serves as a foothold for crack propagation in the cement as a result of mastication. Increasing gap formation may induce secondary caries, bonding failure, restorative fracture in the body or at the margins, and postoperative sensitivity.[9]

Clinical criteria have been used to assess marginal integrity in long-term clinical trials.[29,30] However, these investigations are costly, time-intensive, and often technically challenging to complete. In contrast, simulated wear measurement could provide a rapid means with which to examine relative wear rates among materials and to predict expected clinical performance.[31,32]

In the present study, a sliding-impact wear apparatus was used as a two-body contact wear simulator. In a three-body contact wear model, abrasion, adhesive, and erosion wear are likely the dominant forces, but their relative contributions are not known. However, for a two-body contact wear model, it is considered that abrasion, adhesive wear, and fatigue or attrition are dominant.[33] Although three-body wear testing with an abrasive medium has been used frequently to simulate the mastication of food, the use of abrasive media influences the results as a result of the formation of an embedded layer of abrasive medium and creates a difficulty with standardizing these mixtures or maintaining a consistent viscosity and composition during the entire wear simulation process.[34] These considerations suggest that the two-body wear test employed in the present study has the advantages of eliminating variability and establishing a simplified model.

The wear values (maximum facet depth and volume loss) for the six resin cements appear to be material dependent, as was also determined for the mechanical properties. The self-adhesive cements, apart from GL, demonstrated significantly lower wear resistance than did the control material EC. Therefore, the null hypothesis that self-adhesive resin cements will show significant differences in wear resistance and mechanical properties from the control resin cement was not completely rejected. From the SEM images of wear facets after impact-sliding wear testing, GL and EC revealed relatively flat and smooth surfaces, in addition to some fine cracks on the worn surfaces. On the other hand, the other self-adhesive cements exhibited rougher and deeper facets than did both GL and EC. The other materials also exhibited a similar wear pattern, with some plucking of filler particles from the surface. Considering the impact and sliding force administered by the wear equipment employed, it could be considered that smaller filler particles would preserve the surface texture even if some filler were plucked from the resin matrix and might inhibit crack propagation.[10]

It has been reported[35,36] that improvement in the degree of conversion of resin composites is expected to increase wear resistance and mechanical properties. In addition, previous studies suggested that dual-cured self-adhesive resin cements with photo activation would obtain a superior degree of conversion compared with self-curing alone, because acidic functional monomers act negatively on the self-cure setting reaction by their relatively lower pH value[18,19] and interfere with the reaction between the tertiary amine and camphorquinone.[20-22] Although the specimens for mechanical properties and wear resistance were evaluated after photo activation, it is possible that the acidic functional monomers influenced the polymerization of the self-adhesive resin cements.

While mechanical properties and wear behavior are not the only parameters for consideration in the

selection of resin cements, these characteristics can provide valuable information to dental professionals.

CONCLUSIONS

The results of this study showed that the self-adhesive cements tended to have poorer mechanical properties than the conventional resin cements. For wear resistance, the self-adhesive resin cements, apart from GL, showed significantly lower values than did the conventional resin cements. These results augment the information base available to the profession and provide guidance for clinicians in the selection of resin cements.

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Conflict of Interest

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

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