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President's Award

Barbara Young

Barbara Young

The following are comments made by Dr Barbara Young upon the occasion of her being recognized as The 2016 Presidents Award recipient for the RV Tucker Study Clubs.

Welcome to the homeland of our beginnings – the Pacific Northwest. Seattle has always been a very special meeting place for our Academy.

I'd like to thank Carol Klingensmith for selecting to recognize me tonight. There are so many wonderful members that should be standing up here that I know the task was difficult. Carol comes from a line of amazing President's of this Academy that I have served with during my term as Editor. She will always be special in my heart. During her term as President, we lost our great Mentor, Dr. Tucker. Carol has served the Academy with grace and compassion during such a difficult time. She honors us all.

It's quite an honor to be a recipient of the President's Award.

There's no feeling like it. Do you melt or do you glow? I guess a little of both because it is humbling.

I'd also like to thank the Executive Council for allowing me to serve as Editor for the Lineangle Newsletter. Thank you Margaret Webb for nominating me. Thank you Jim Deckman, who preceded me as Past Editor – for scratching out on a napkin everything I needed to know, during a clinical critique session in Vancouver. Thank you Dave Thorburn for guiding me. Thank you Warren Johnson for honing my understanding of what it takes to "make it shine."

I was never at a loss for the next story. The many articles each of you submitted filled the pages. Bringing such a diverse Academy together became great fun for me. Whenever I came to a fork in the road and had to make a decision on how to make the issue different and more interesting, I had to decide whether I should I go to the right or to the left. Each turn was something new and unknown. But, what I didn't know was that the roads converged as one again, as with many things represented by this Academy.

I'll let you in on a secret I learned from Dr. Tucker, Warren Johnson, Rich Stevenson, Ted Kanamori, and my wonderful husband, Dr. Stan Siegel; They have taught me how to better "Honor my Life." I in turn, make it a point to honor someone everyday. Your trademark is your honor.

Tonight I'd like to honor my Family and my Peers —

Past Presidents of this Academy during my term as Editor:

Randy Allen, Barry Evans, Bruce Small, Andreas Bien, Maureen Andrea, Manuel Cordero, Dana Otterholt, and Carol Klingensmith.

There's one more very, very special person I need up here:

Ron Gusa - The Academy's creator and first Editor of the Lineangle Newsletter. Thank you Ron.

As these people stand, I want you to know what DR. TUCKER said to me once, in his easy going way:

"There's nothing like it..... It's a profession where... it's amazing how people from all over the world become such good friends."

"There's really nothing like it anywhere."

I would like each of you to know how amazing you are.

And when I look at this plaque, know that I share this honor with you.

Bravo!!!

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Clinical Technique/Case Report

Conservative Anterior Partial Coverage CAD/CAM Restoration

JT May

SUMMARY

Computer-aided design and manufacturing technology enables practitioners to create, in a single appointment, indirect restorations that are esthetic and functionally unique to the patient's situation. The popular effort to perform minimally invasive dentistry using digital techniques with chairside milling can lead dentists to novel individualized restorative treatment. This article demonstrates a conservative anterior partial coverage restoration, utilizing both digital technology and chairside ceramic characterization to achieve an optimal esthetic outcome while preserving healthy tooth structure.

INTRODUCTION

Single-appointment computer-aided design and manufacturing (CAD/CAM) dentistry has opened a new treatment option for restoring teeth with an indirect chairside approach. The effectiveness of digital scanners are shown to be at least as exacting and reliable as more traditional impression techniques.[1] Numerous studies have demonstrated long-term success with various CAD/CAM restorations, with most citing a success rate of approximately 90% at 10 years.[2-5]

Conventional treatment for moderately to severely broken-down anterior teeth having nonsurgical root canal therapy (NSRCT) typically includes direct resin composite buildup; resin composite core and full-coverage crown; prefabricated post, resin composite core, and full-coverage crown; and custom cast post/core and crown. Unless the access of the NSRCT was minimally prepared, very little tooth structure remains to support the core substructure and the subsequent crown. In addition, because the enamel thins as it approaches the cemento-enamel junction, cervical dentin is typically exposed, leaving minimal enamel bonding available. This is especially true with subgingival margins. This places all of the retentive stress on the post, core, and bond between the dentin and crown material. A lack of natural tooth structure weakens the tooth and increases the chance of the crown failure, making further treatment more difficult or invasive. In addition, the typical crown preparation margin is placed slightly subgingival to improve esthetics or in an effort to increase the ferrule effect.[6,7] In terms of bonding substrate, adhesive bonds to enamel are shown to be stronger and more durable than bonds to dentin.[8,9] Therefore, maximizing preparation surface area in enamel should increase restoration performance and longevity.

The trend in modern dental practice is toward a more conservative approach that preserves natural tooth structure while still providing long-lasting esthetic restorations.[10-13] By minimizing weakened tooth structure, removing existing restorative material, and keeping preparation depth in enamel, a greater amount of tooth can be preserved. With careful attention to tooth preparation, restoration design, and a quality custom stain, a very esthetic and strong partial coverage ceramic restoration can be delivered without sacrificing supportive structures. The purpose of this case report is to present an

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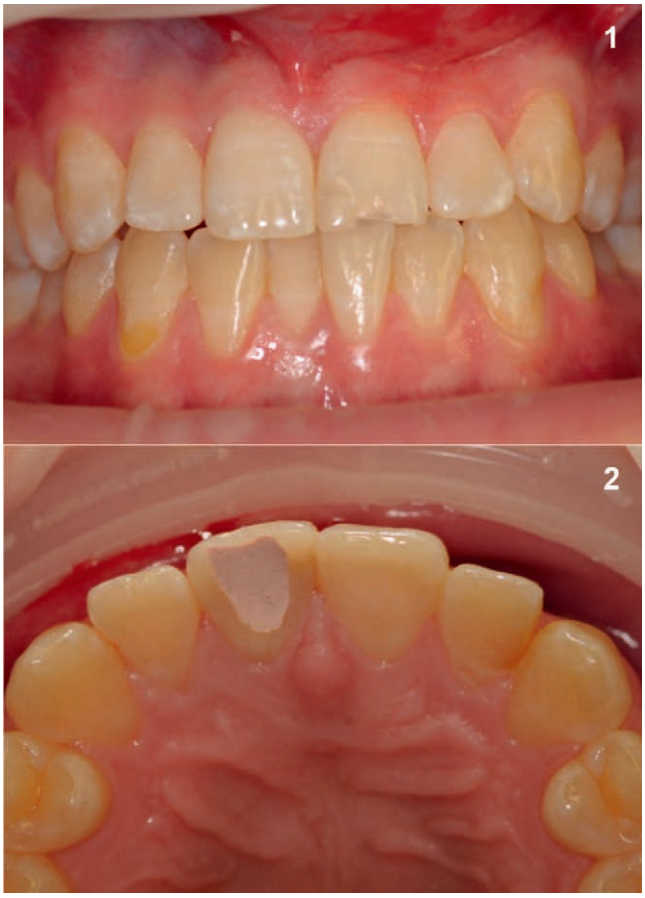

Figure 1. *Preoperative facial photograph showing fractured incisal edge and existing MIFL composite.*
Figure 2. *Large endodontic access with provisional restoration.*

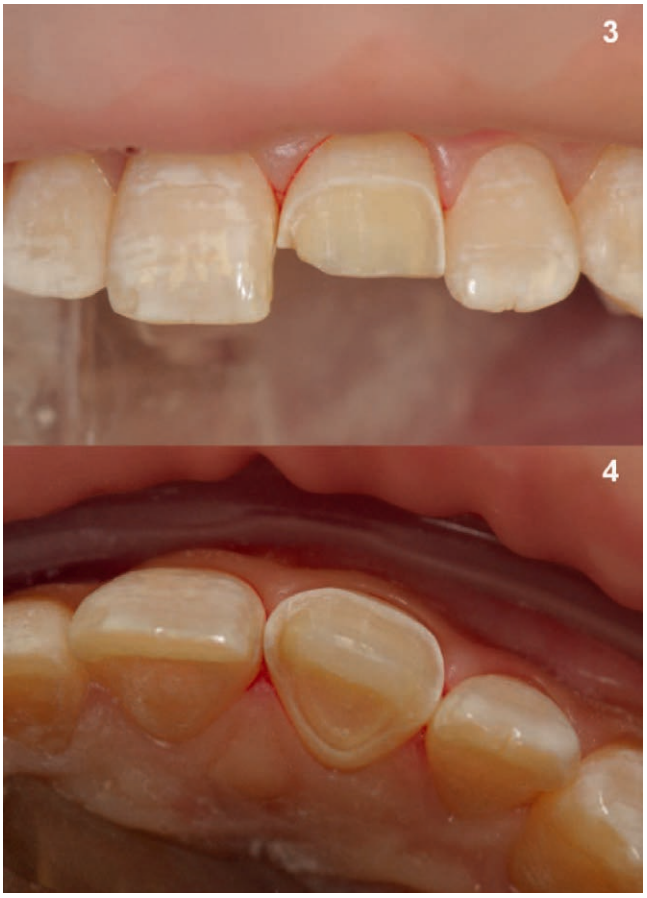

Figure 3. *Anterior preparation, including finish line placed just below the height of contour.*
Figure 4. *Incisal view of the preparation. Notice that the lingual finish line was left at the previous access margin and the thick band of enamel 360 degrees around the preparation.*

alternative chairside CAD/CAM approach to traditional maxillary central incisor crown preparation utilizing a conservative esthetic preparation design.

CLINICAL TECHNIQUE REPORT

The patient presented with a provisionalized NSRCT retreat on #9 with a history of trauma, which led to the previous NSRCT, and multiple composite repairs (Figures 1 and 2). The patient was referred for an esthetic full-coverage restoration. During the evaluation, the patient explained he was not impressed with the previous composite restorations because they did not reproduce the fluorosis staining of the surrounding dentition. Options for treatment were discussed with the patient, including a composite buildup; core and crown; post (prefabricated or cast), core, and crown; and a partial veneer CAD/CAM all-ceramic restoration. The option of a CAD/CAM lithium disilicate partial veneer restoration was selected. Lithium disilicate has been increasingly used in thin veneer restorations due to impressive strength and esthetics.[10-14]

The advantages of this treatment option are numerous. With the large endodontic access, a traditional crown would be supported almost entirely by the post and core rather than natural tooth structure. A conventional crown preparation would sacrifice almost all of the remaining coronal tooth structure, further weakening the tooth.[15] Additionally, the patient was concerned about the possibility of receiving another potentially unesthetic composite restoration. Due to the amount of enamel remaining, the patient's preference for a less invasive treatment option, and the high esthetic demand, a conservative partial all-ceramic veneer restoration was selected. Enamel bonding is very predictable, while dentin bonding varies greatly based on type of bonding agent and type of tooth and between different patients.[12] In addition to these factors dentin

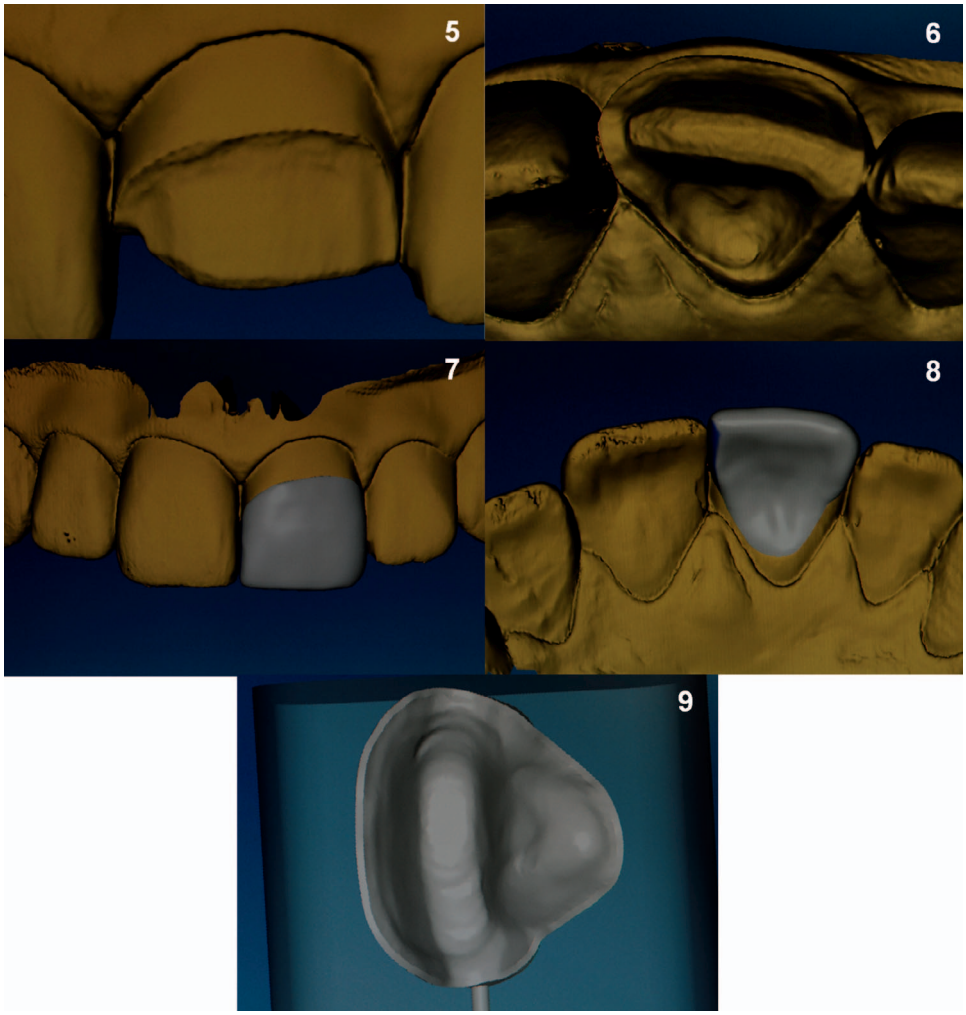

Figure 5. *Facial view of the preparation.*
Figure 6. *Incisal view of the preparation.*
Figure 7. *Image of the proposal.*
Figure 8. *Lingual of the proposal.*
Figure 9. *Intaglio of the proposed restoration. Notice the very small amount of overmill and the added 200-micron addition to the margins to protect the margin during the milling process.*

bonding has been shown to degrade over time.[16-18] Based on clinical findings, this option maintained the majority of circumferential enamel, allowing bonding of the most ideal substrate for long-term retention and success.

The patient was anesthetized (Septocaine [Articaine HCL 4%], Septodont, Lancaster, PA, USA), and the anterior dentition was isolated (Optragate, Ivoclar Vivadent, Amherst, NY, USA; Isodry, Isolite Systems, Santa Barbara, CA, USA). The provisional restoration, cotton pellet, and original composite restoration were then removed. The self-etch resin adhesive was applied (Clearfil SE Protect, Kuraray, New York, NY, USA), and a universal nanofilled resin composite (Premise, Kerr, Orange, CA, USA) was placed in 2 mm incremental layers to complete the core substructure.[19,20] The facial surface was prepared using 0.5 mm veneer reduction burs (0.5 mm depth cutter diamond, Neodiamond Microcopy, Kennesaw, GA, USA). The remaining tooth was then prepared with a minimal preparation using rotary diamond cutting instruments (coarse and fine diamonds, round-end taper and flat-end taper, Neodiamond Microcopy) (Figures 3 and 4), and the digital impression was captured (CEREC Bluecam, Sirona, Charlotte, NC, USA) (Figures 5 through 9). The cervical lingual margin of the endodontic access was used as the finish line for the restoration, and proximal contacts, with the cingulum, were maintained to the greatest extent possible. This design allows for conservative preparation to retain enamel and create a light chamfer finish line.[11] Milling a light chamfer can be problematic and result in chipping if the margin is too thin. In order to prevent this, the restoration margin thickness parameters were changed from the 50 micron manufacturer recommendation to 200 microns. This change created a small amount of overlap at the margins to allow for sufficient bulk. This excess, which was trimmed and polished prior to crystallization, allows the mill to create an accurate fitting restoration without risk

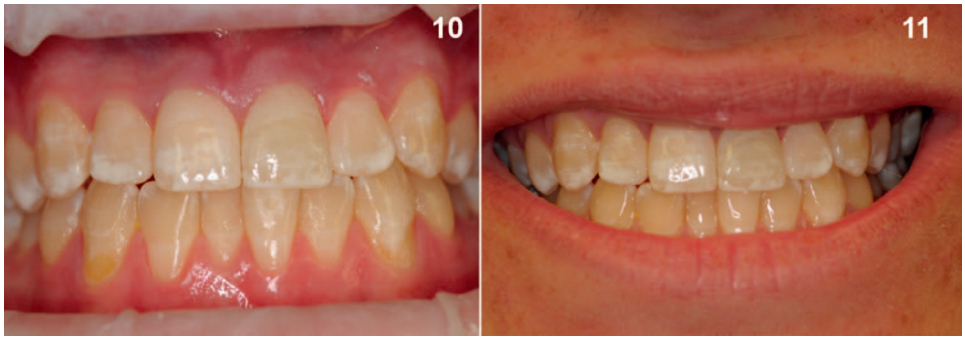

Figure 10. *Delivered #9, custom stained and bonded in place.*
Figure 11. *Final smile after delivery.*

of chipping the margin. The incisal region of the preparation was minimally flattened to accommodate the size of the milling bur, reducing the possibility of overmill.

The restoration was milled (CEREC MC XL, Sirona) out of an A1-HT-12 (high-translucency) lithium disilicate block (IPS e.max CAD, Ivoclar Vivadent). While still in its lithium metasilicate state, the restoration was tried in and adjusted accordingly using light pressure with a finishing flame diamond and copious water. The margins were repolished using an extraoral finishing and polishing system (Dialite LD, Brasseler USA, Savannah, GA, USA). The restoration was then prepared for crystallization, and a custom stain and glaze (IPS Empress Universal Shade/Stains, Ivoclar Vivadent) were applied to re-create the white characterization and striations. After crystallization, the restoration's fit was verified, and the esthetics were approved by the patient. The conservative 2/3 partial veneer CAD/CAM crown was bonded using a yellow dual cure resin cement (Variolink II, Ivoclar Vivadent) (Figures 10 and 11). Completion of the restoration included excess cement removal, occlusion adjustment, and marginal repolishing (Dialite HP, Brasseler USA).

DISCUSSION

The CAD/CAM partial coverage restoration presented here maximized the conservation of enamel and coronal tooth structure while providing full support and incisal coverage after endodontic retreatment. The patient's esthetic expectations were met while maximizing sound tooth structure.

Understanding how CAD/CAM technology can be applied allows for modern restorative treatment options to be more conservative than some traditional approaches. The ability to fabricate strong custom-stained lithium disilicate restorations chairside provides the clinician with an efficient and versatile procedure, eliminating the need for a return visit for most patients.

The author selected the 200 micron change due to personal experience and preference. It is possible that this is overly cautious and that a smaller margin thickness parameter may be satisfactory. This is one of the advantages of a CAD/CAM system; namely, it allows the practitioner to adjust restoration parameters to personal preferences.

As CAD/CAM technology becomes increasingly integrated into restorative dental practice, the ability of the practitioner to create and control the fabrication of unique indirect restorations will expand.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Marine Corps Recruit Depot Parris Island, Branch Dental Clinic.

Navy Disclosure

The opinions or assertions contained in this article are the private ones of the author and are not to be construed as official or reflecting the views of the Department of the Navy.

Conflict of Interest

The author of this manuscript certifies that he has 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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A Direct Refractory Die Technique for Cast Gold Restorations

CT Smith • KE Diefenderfer

Clinical Relevance

This direct refractory die technique eliminates wax pattern distortion and facilitates the creation of accurate, precisely fitting cast gold restorations.

ABSTRACT

Fabricating accurate cast gold restorations can be challenging for both clinicians and laboratory technicians. Removing the wax pattern from the master die often distorts the pattern, which, in turn, compromises the overall fit and marginal adaptation of the casting. This article demonstrates a laboratory technique in which the final restoration is cast directly on the refractory die without removing the wax pattern. Thus, distortion of the wax pattern is avoided, enabling the production of superbly fitting gold castings for both intracoronal and extracoronal restorations.

INTRODUCTION

Producing cast gold restorations that are consistently precise and well fitting can be a challenge for both clinicians and dental laboratories. Contributing to this challenge is the issue of wax pattern distortion during the fabrication process.[1-3] Distortion of the wax pattern during removal from the master die and investment is "unavoidable."[3,4] However, use of refractory die techniques can greatly reduce this problem if carefully employed in the process.[5-7] Refractory die techniques have been used for the laboratory fabrication of various dental appliances and restorations for many years.[8-11] Currently, however, a precise and consistent fabrication technique for cast gold restorations on refractory dies has remained elusive and underutilized in restorative dentistry.

Described here is a clinical and laboratory technique for fabricating extremely accurate, smooth, and detailed gold castings for both intracoronal and extracoronal restorations.

CLINICAL PROTOCOL

After the tooth preparation has been completed (Figure 1), the gingiva is retracted with a hemostatic-impregnated cotton cord and the preparation cleaned of all debris, then air-dried. A rigid metal check-bite tray (GC America, Alsip, IL, USA) with perforated sidewalls is tried in the mouth. All large open embrasure spaces and undercuts should be blocked out with wax or some other material (in the quadrant to be impressed) prior to the impression procedure. This will minimize the possibility of locking and distorting the impression.

The preparation is impressed with a light/heavy-bodied polyvinyl siloxane impression material sys-

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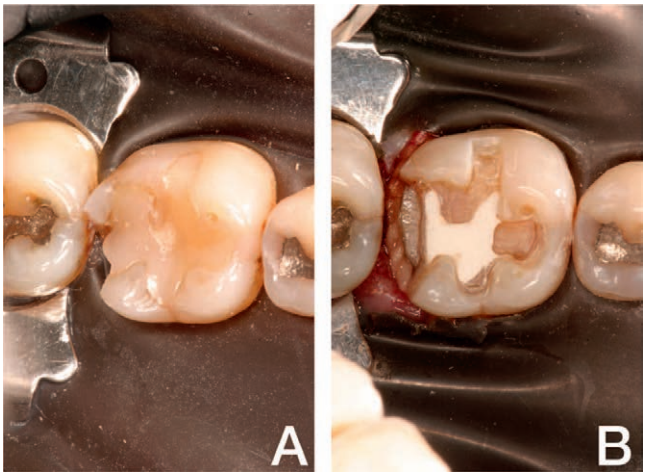

Figure 1. *Number 19 defective resin composite with secondary caries. (A): Pretreatment. (B): Completed cast gold onlay preparation. The DB and DL cusps were judged to have sufficient dentin support to warrant conservative preparation. Note that the interseptal rubber dam has been cut to enable retraction cord placement with the dam in place.*

tem (Aquasil [Dentsply/Caulk, York, PA, USA] is recommended) in the usual manner, ensuring that the patient closes into maximum intercuspation. Support the patient's mandible in the closed position with the loaded impression tray in place. The impression is allowed to set according to the manufacturer's recommendations, but verify that the material exhibits "total rebound to blunt depression" before removing from the mouth.

On removal from the mouth, the impression is rinsed with a surfactant solution (Harvest Surfactant, Harvest Dental, Brea, CA, USA) and dried thoroughly with air. Make sure all the preparation finish lines are well defined and visible, with no bubbles or distortions present in this area (Figure 2).

LABORATORY TECHNIQUE

Die Preparation

The impression is now taken to the laboratory for pouring and mounting. Undercuts or excess impression material are removed with a sharp surgical blade prior to boxing of the impression. The impression is boxed with a polyvinyl siloxane putty on the mesial and distal portions of the tray (Figure 3) to prevent the refractory investment from spilling out over the ends of the tray.

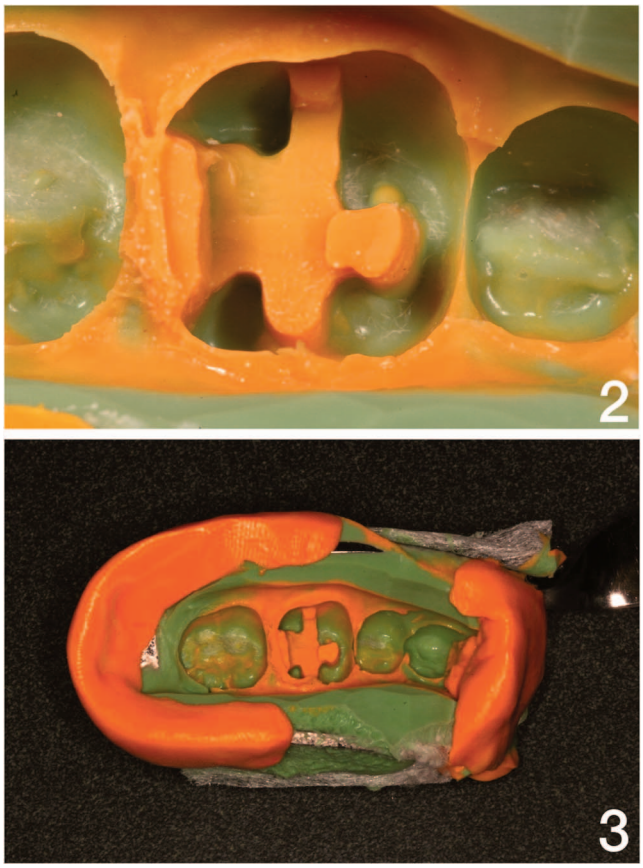

Figure 2. *Final impression must be well defined and clean.*
Figure 3. *The impression is boxed with putty on the mesial and distal of the tray.*

To create the refractory die, the impression is poured using a high heat micro-fine phosphate-bonded investment material (Starvest, Emdin International Corporation, Irwindale, CA, USA) rath-

Table 1: *Powder-to-Liquid Ratios for Gold Castings*[a]

Casting	Starvest Powder (g)	Starvest High-Expansion Liquid (g)	Distilled Water (g)
Inlays (one to four surfaces)	50.000	14.250-14.500	0.400
Onlays and crowns (partial or full)	50.000	14.750-15.000	0.400
Post and cores	50.000	13.850-14.250	0.400

[a] *The ratios may need adjusting according to the lot number of the investment being used at the time. Different lots of the investment can vary in physical characteristics and may have different expansions. Adjust as needed by increasing or decreasing the weight of the expansion liquid (an increase of expansion liquid will cause more expansion of the casting). Keep the weight of H_2O constant. Small incremental changes of the high-expansion liquid can make significant changes in expansions of the castings.*

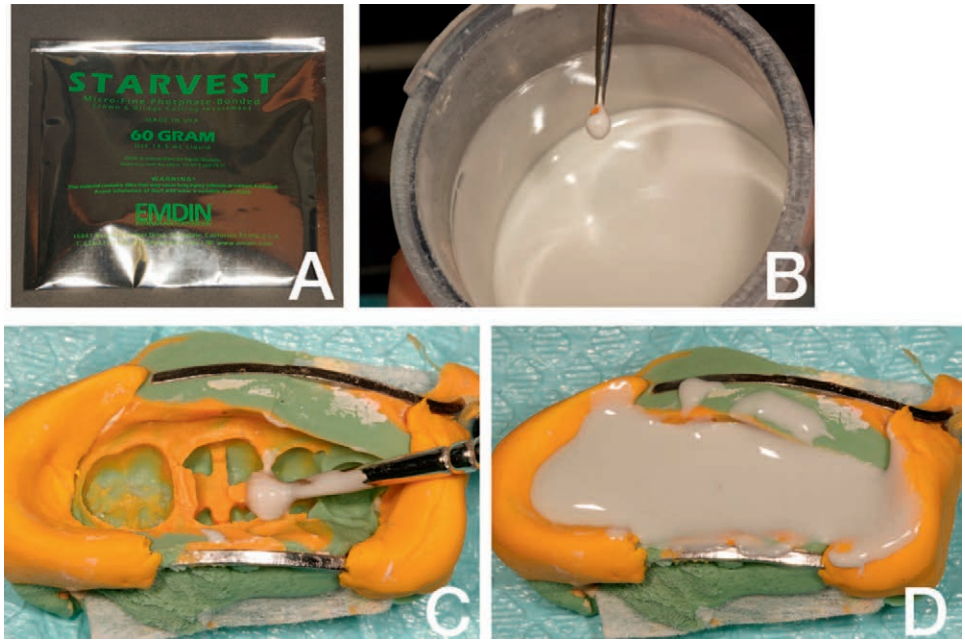

Figure 4. *Starvest phosphate-bonded investment material (A) mixed under vacuum (B) and poured into the impression (C and D).*

er than conventional die stone. This finely ground investment has a powder particle size of 5-7 microns and produces a smoother cast surface, both internally and externally, than was possible in the past with other investments. The older refractory investment powders had a larger particle size, which may result in rougher surfaces and less precise castings.[12] Exactly 50 g of investment powder are weighed out in a plastic container. A predetermined weight of high-expansion colloidal silica mixing solution (Emdin High-Expansion Liquid, Emdin International), combined with a corresponding weight of distilled water, is used for specific preparation configurations (Table 1). The liquid components are weighed to 0.001 g in a tared vacuum-mixing bowl on a digital analytical scale.

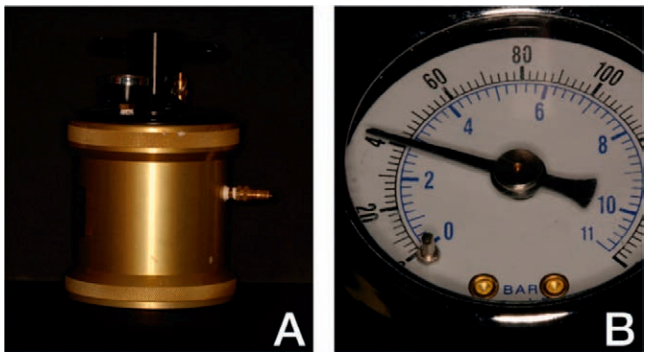

Figure 5. *(A): Lang Investpress. (B): Pressurize to 40 psi.*

The investment powder is added and the mixture spatulated in a digital vacuum mixer under vacuum for 60-90 seconds at 450 rpm.

The investment is carefully poured and vibrated into the impression. A small soft brush can be used to paint investment into the preparation (Figure 4); this may be helpful in avoiding bubbles on the refractory die and adjacent surfaces. The remaining investment is added until the preparation is covered with 3-5 mm of investment. The poured impression is immediately placed in a dry pneumatic pressurized curing unit (Investpres, Lang Dental Manufacturing Co, Inc, Wheeling, IL, USA) at a pressure of 40 psi for 30 minutes (Figure 5). Allowing the investment to set under positive pressure results in improved surface smoothness, with fewer and smaller surface voids in the set cast.[13,14] Moreover, in our experience, the refractory dies are more resistant to abrasion by metal waxing instruments, and the subsequent castings appear to be smoother, enabling more efficient polishing.

Following complete setting, the poured checkbite tray impression is mounted on a quadrant articulator (Monotrac V2, Monotrac Articulation, Midvale, UT, USA). The articulator base is first lubricated with a silicone separating medium (MS3 Master Separator, Harvest Dental, Table 2). Next, a second batch of investment material is mixed to a thicker consistency and poured into the articulator

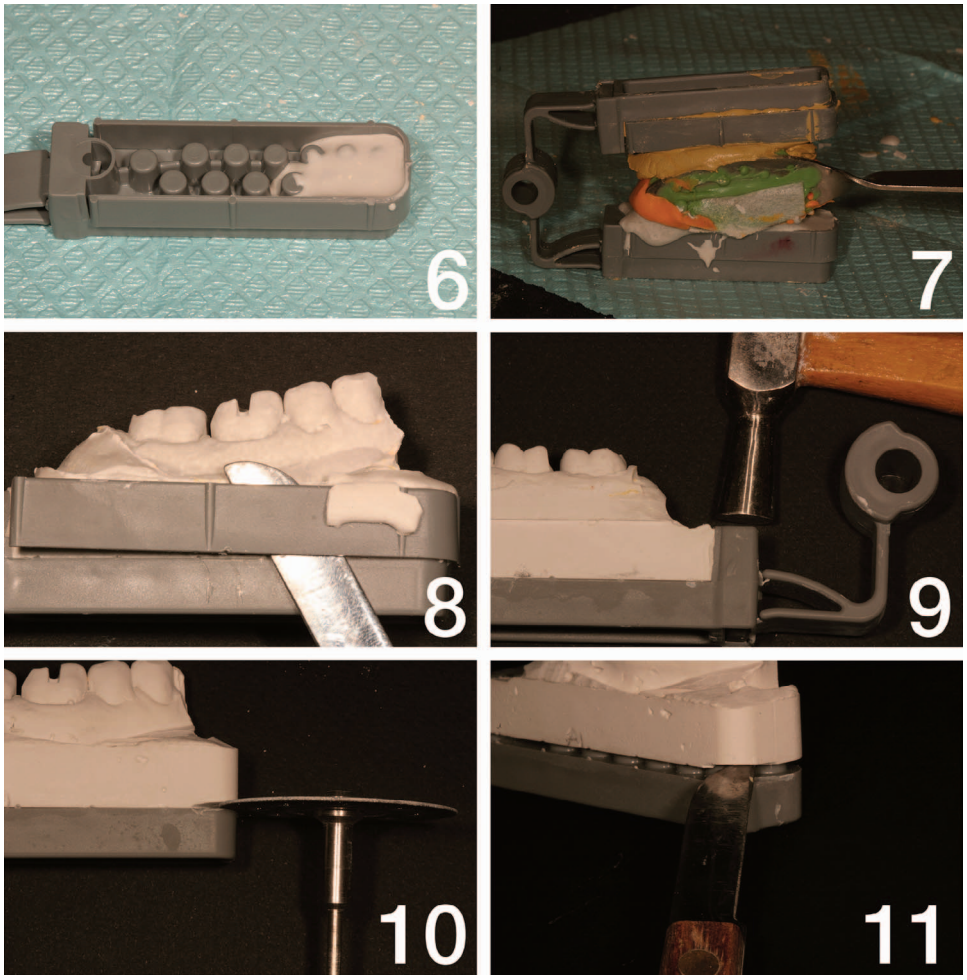

Figure 6. *Monotrac articulator base with a thick mix of investment.*
Figure 7. *Opposing arch poured and mounted after the working arch hardens.*
Figure 8. *Remove the flash and plastic form from the articulator.*
Figure 9. *Lightly tap the base until a small space is formed.*
Figure 10. *Use a separating disc to create a slot in the base.*
Figure 11. *Gently separate the cast from the articulator base.*

base (Figure 6); exact measurement of the liquid-to-powder ratio is not necessary for this step. A portion of this mixture is placed on the exposed hardened side of the investment in the impression. The check-bite tray impression is now placed on the articulator base and stabilized for 30 minutes for hardening. The opposing arch of the impression is then poured in a conventional die stone (Figure 7).

Separation of the refractory model from the impression should be done carefully. First, remove

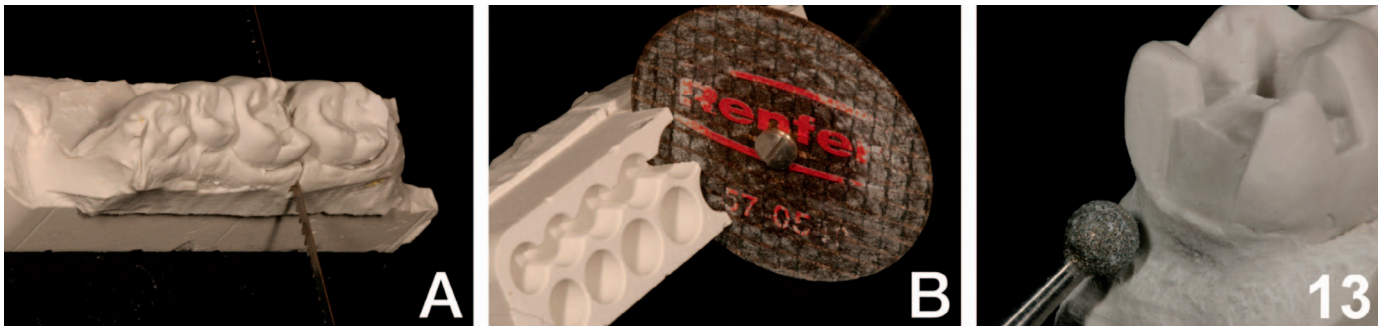

Figure 12. *(A): Make a 4-5-mm-deep cut with a die saw on the proximal. (B): Section the remainder of the base with a separating disc.*
Figure 13. *Trim the die to expose the preparation finish lines and tooth emergence profiles.*

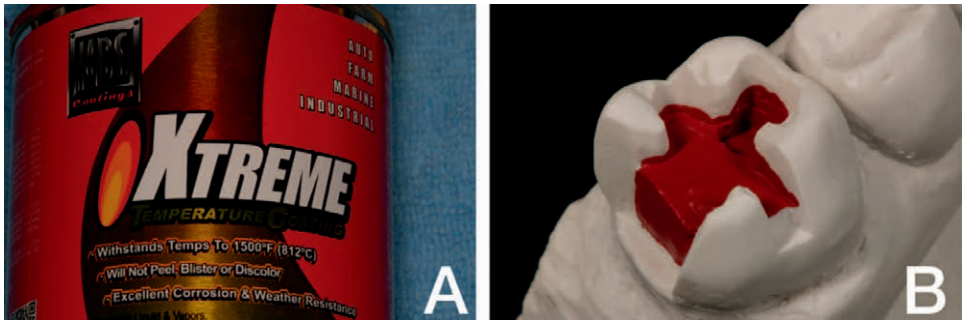

Figure 14. *(A): High-temperature paint for the die spacer. (B): Application of two to three coats creates a matte finish.*

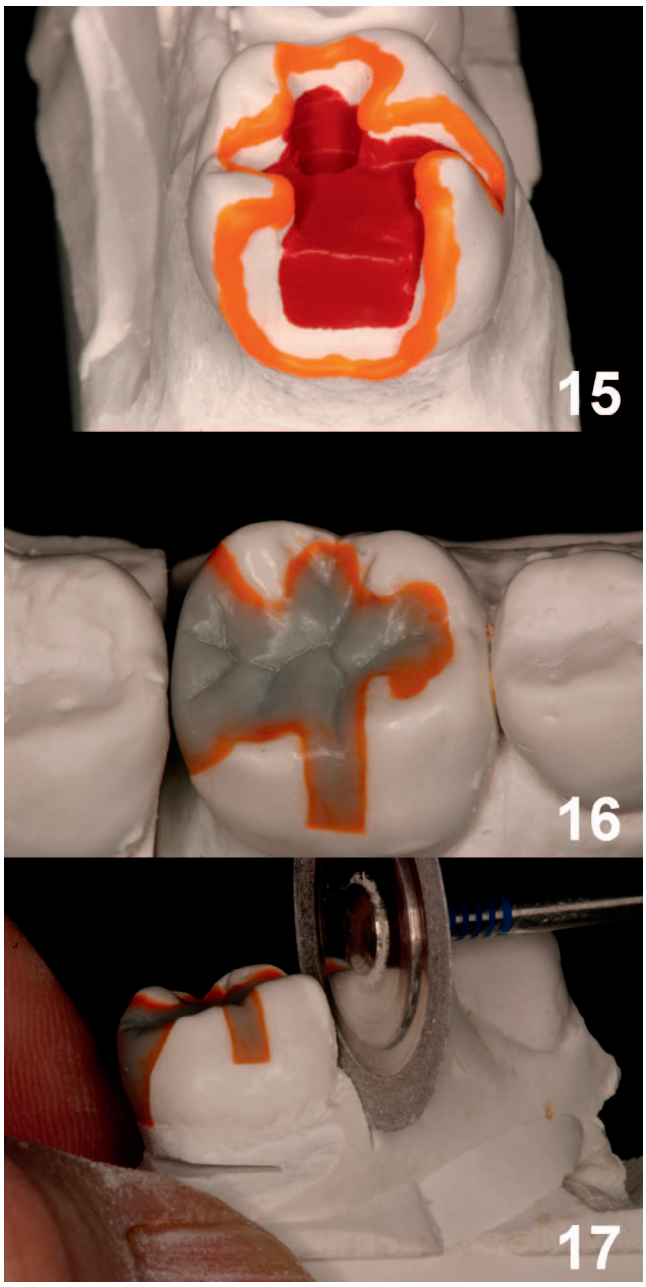

Figure 15. *Contrasting bright opaque wax margin is applied first.*
Figure 16. *Final wax-up.*
Figure 17. *Section the refractory die and wax pattern from the base.*

the distal of the impression tray from the refractory investment die/base. This will decrease the potential for distortion of the metal tray and impression. Break off the excess refractory investment and plastic form from the articulator (Figure 8). Tap with a small hammer lightly on the plastic base near the articulator hinge to create a thin space between the refractory investment base and the plastic base (Figure 9). Next, use a large diamond separating disc (Brasseler USA, Savannah, GA, USA) to place a horizontal slot in the front of the refractory die base where the plastic base and the investment meet (Figure 10). Insert a Buffalo knife blade into the slot and gently rotate back and forth to loosen and separate the refractory die base from the articulator base (Figure 11).

Remove the refractory die base from the articulator base. Make a 4-5-mm-deep saw cut in the proximal(s) (Figure 12A) and section the remaining portion with a diamond- or glass-reinforced separating disc (Dynex disc, 40 mm × 0.5 mm, Renfert, Hilzingen, Germany) from the underside of the base (Figure 12B). Trim the die with a mounted diamond stone and appropriate instruments to expose the preparation finish lines and tooth emergence profiles (Figure 13). Clean dies and models with pressurized air to remove all refractory residue.

A high-temperature automotive paint (XTC, KBS Coatings, Valparaiso, IN, USA; Figure 14A) is an ideal die spacer. Mix 10%-20% refractory investment powder thoroughly into the paint and apply to the appropriate surfaces (pulpal floor and axial wall[s] for inlays; pulpal floor, axial walls, and occlusal portion for onlays; and the occlusal and axial walls for extracoronal preparations]. Two or three coats should be applied and allowed to dry completely; this should produce a smooth matte rather than a glossy finish (Figure 14B) and should provide approximately 25 microns of die relief, which is adequate space for the luting agent (the material can also be used as a block out for undercuts). It is not always necessary

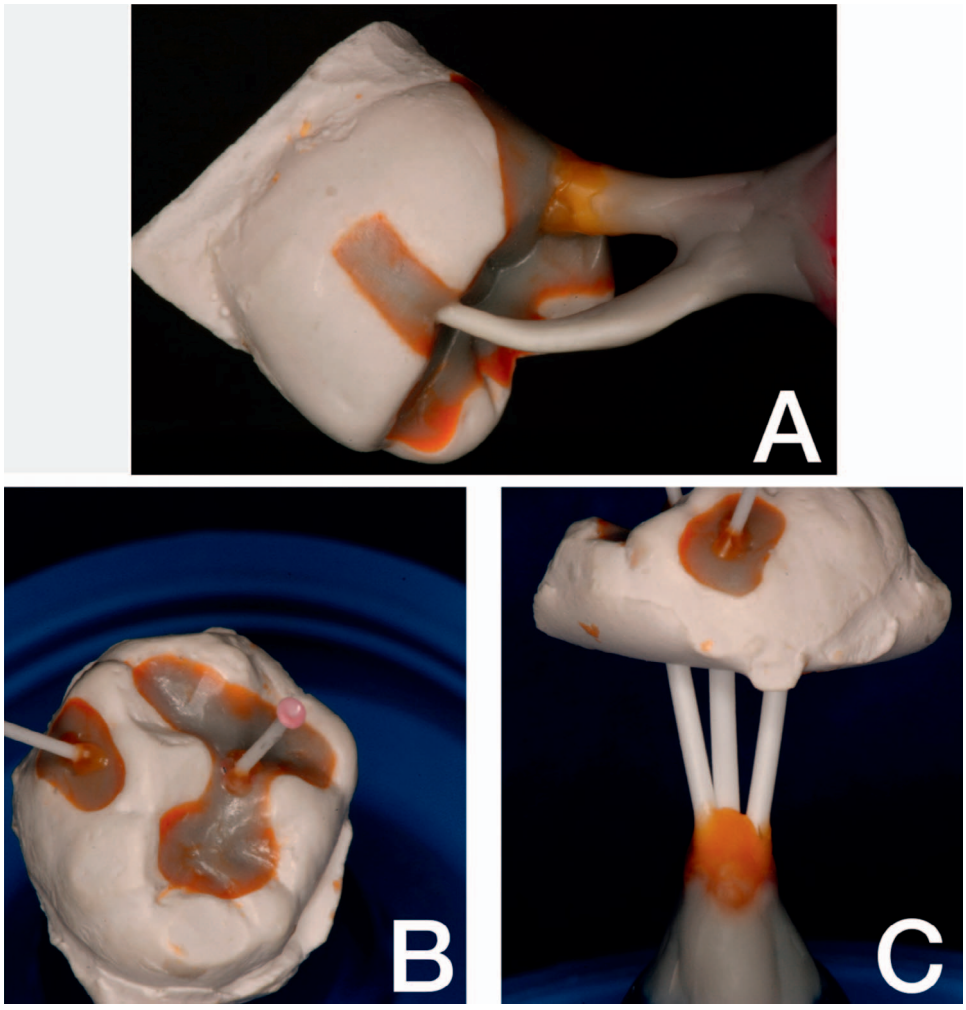

Figure 18. *(A): Sprue the wax pattern and refractory die. (B and C): Sprue through the die for one-surface inlays.*

to place a die spacer in refractory inlay dies. When carefully executed, manual relief of the inlay casting axial walls and pulpal floor with a white stone can provide space for a luting agent and relieve undercuts on cast inlays.

Wax-Up

Begin the wax-up by applying a thin line of bright-orange–colored contrasting wax (Consequent, Yeti Dental, Engen, Germany) to all finish lines of the preparation (Figure 15). This is a block-out wax, but works well to highlight the margins during the wax-up of the restoration. The refractory die material is very white, and most buildup waxes are too translucent to enable detection of overextended wax margins. This results in castings with overextensions of gold on the margins. Next, apply a sculpting wax (Thowax, Yeti Dental) to the remainder of the die. Finalize all external contours of the wax pattern. Remove all excess wax at the cavosurface margins with a wax-carving instrument (DPT Carver, Hu-Friedy, Chicago, IL, USA). Gently smooth the wax pattern with an artificial soft sable brush and carefully polish the axial surfaces and margins with a microfiber lens cleaning cloth to finish the wax pattern (Figure 16).

Investing the Wax Pattern

Using a diamond- or glass-reinforced separating disc, section and cut the refractory die, with its wax pattern in place, from the base 3 mm below the existing margins (Figure 17). Keep the wax pattern and die free of the refractory die residue by frequently cleaning with pressurized air. If refractory residue is left on the wax pattern, a rough surface will be produced on the casting. The wax pattern is sprued on the contact areas or cusps of full crowns, onlays, and multiple-surface inlays

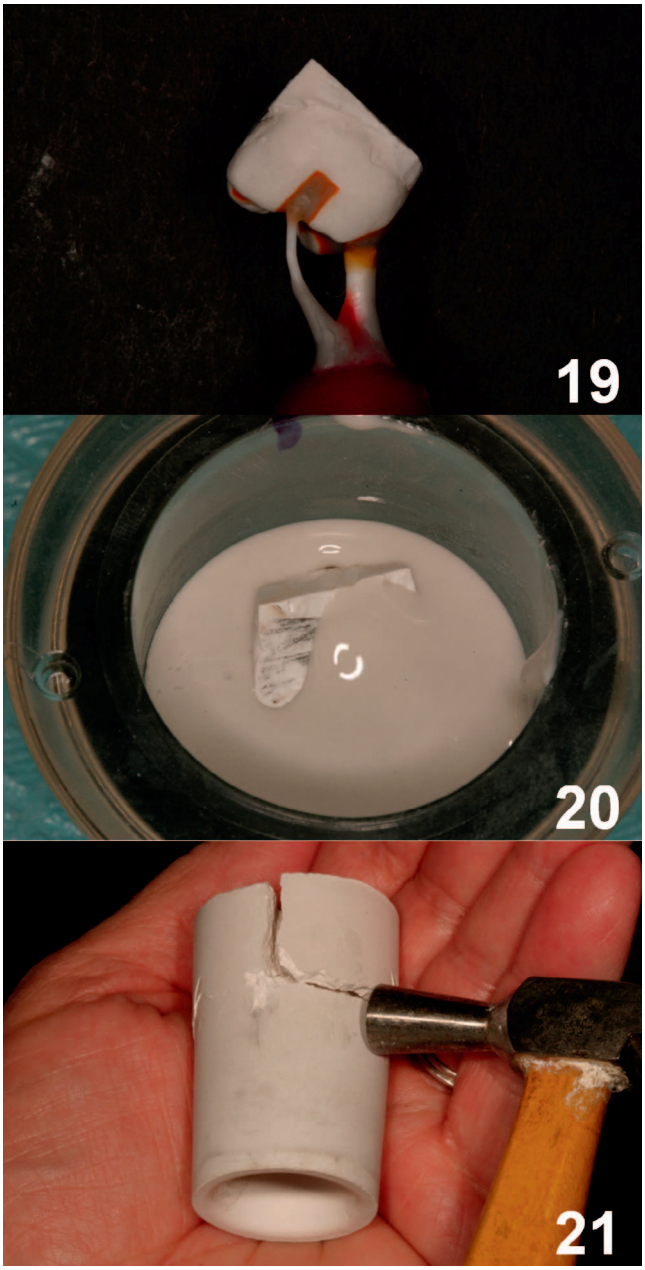

Figure 19. *Attach the wax pattern and refractory die to the sprue base.*
Figure 20. *Cover the refractory die and wax pattern with a second (identical) mix of Starvest phosphate-bonded investment.*
Figure 21. *Break out the casting from the investment.*

(Figure 18A). Single-surface wax patterns are often sprued through the refractory die to the bottom of the wax pattern (Figure 18B). This is accomplished by boring a small hole(s) through the underside or side of the refractory die to the underside of the wax pattern. Attach the wax pattern/refractory die to a 3-5-mm length on a sprue base (Figure 19), then place the investment forming ring on the sprue base, orienting the angle of wax pattern/refractory die to be in the trailing position of the centrifugal casting machine's rotation. Index this position on the ring former and the hardened investment for orientation in the cradle of the centrifugal casting machine.

The wax pattern/refractory die is now invested, using the same phosphate-bonded investment material (Starvest) as used for the refractory die. Mix this investment using the same liquid:water:-powder ratio and mixing protocol as used to make the refractory die. If the component proportions differ from that of the refractory die, the expansion rates of the two investments will differ, possibly producing cracks in the investment. Gently vibrate and pour the investment into the ringless investment ring at an angle, covering the wax pattern/die with 3-5 mm of investment (Figure 20). Place the ring assembly into a dry Lang Investpres to a pressure of 40 psi for 60 minutes. Setting of the investment material under positive pressure may result in the production of fewer surface irregularities in the final casting.[15] Remove the ring assembly from the Lang Investpress and remove the base and plastic ring former from the hardened investment ring.

Burnout and Casting

Check to see that no flakes or residue of investment are embedded in the sprue wax button on the underside of the investment ring before placing the investment ring into a calibrated room-temperature burnout furnace. Place the orientation mark on the investment facing toward the oven door. Set the temperature rate at 7°F per minute to rise to a final temperature of 1350°F. After reaching the temperature of 1350°F, hold the temperature for 30 minutes before casting. The total burnout time is approximately three hours and 30 minutes.

Cast and allow the invested casting to bench cool before divesting. Do not divest the casting with an air abrasion unit. Break out the casting with a small hammer in the palm of the hand or on a soft surface (Figure 21). Place the casting in a plastic sealed container containing a solution of 38% hydrofluoric acid or substitute hydrofluoric acid (Figure 22). Place the container in an ultrasonic cleaner and vibrate until the investment is dissolved away. Check the casting under magnification for any bubbles, undercuts, and artifacts; these can be carefully removed with a white stone.

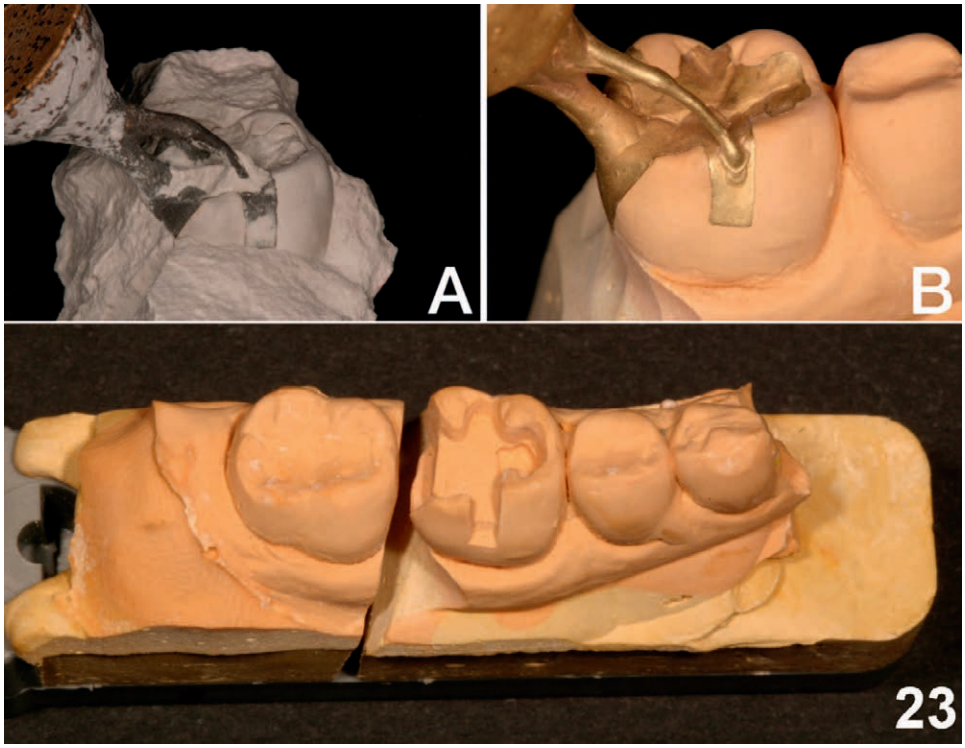

Figure 22. *Casting before (A) and after (B) acid divestment.*
Figure 23. *Working die.*

A second pour of standard die stone is made to fabricate the working master cast for fitting and finishing of the casting (Figure 23). GC Fujirock golden tan (GC America) is the preferred material for this important step because it produces extremely accurate dies consistently across product lot numbers, as verified by evaluations using machined metal standardization dies (C. T. Smith, unpublished data) and its proven record of success over years of use in R. V. Tucker Study Club sessions. Mix 30 g of stone with 6.4 g of distilled water for 30 seconds under vacuum using a digital vacuum mixer. Pour and mount the impression on a Monotrac Articulator. After the stone has set, separate the master model from the impression. Section and trim the master die to expose margins and emergence profiles as needed. The casting should be gently tried on the master die. Evaluate the casting and master

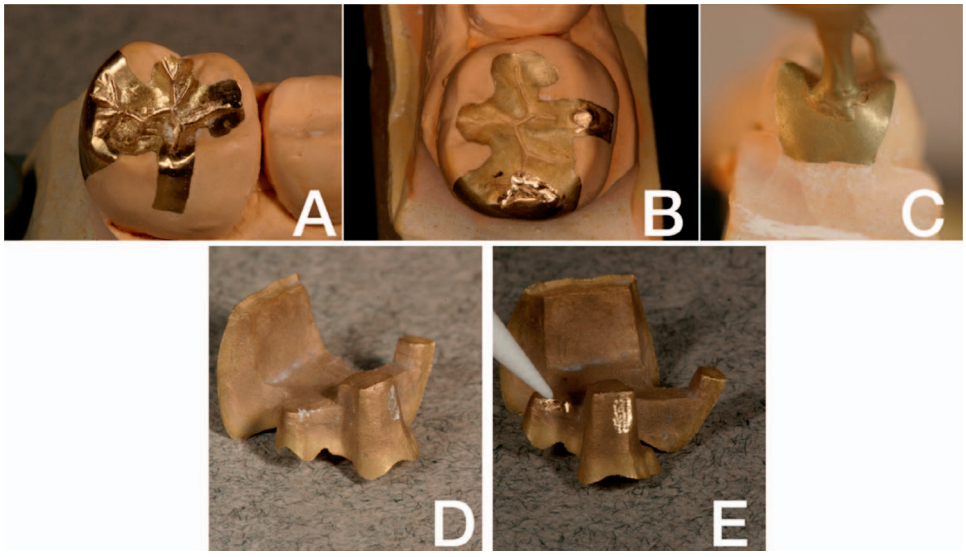

Figure 24. *(A-C): Try the restoration on the working die. (D and E): Adjust the rubs and pressure points.*

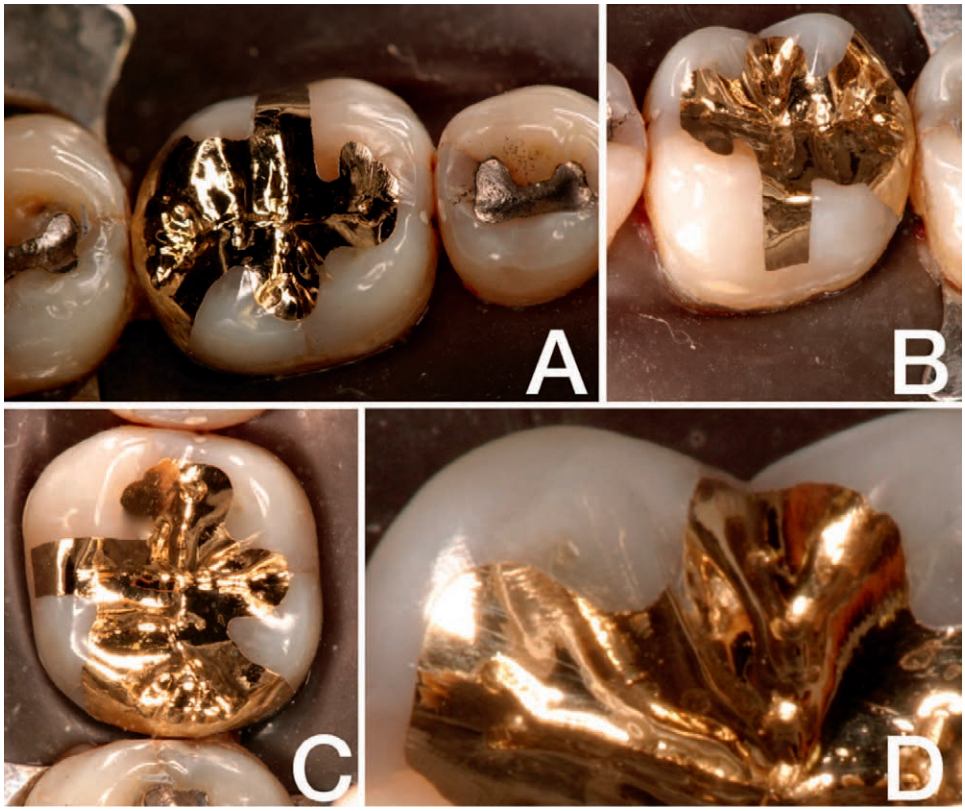

Figure 25. *(A): Initial try-in of the casting before cementation. (B-D): Finished restoration.*

die for minute rubs or powder streaks, which indicate small undercuts or pressure points on the surfaces of the casting and die. These areas on the casting are relieved with the Shofu Dura-White stone (CN1 HP) and lightly abraded with a 27-μm aluminum oxide air abrasive. The casting is placed again on die with gentle pressure, and the sequence of fitting, relieving, and air abrading of the internal surface of the casting is repeated until the casting seats completely and passively on the master die (Figure 24).

When the casting has been completely seated on the working model die, the proximal contact(s) can now be adjusted to final form and fit. The final finish and polish are completed. The casting is now ready for clinical try-in, finishing, and cementation procedures (Figure 25). Table 2 lists the materials and equipment used throughout these procedures.

SUMMARY

The clinical and laboratory techniques described here result in accurate, precise-fitting, and smooth gold castings. Distortion of the wax pattern has been eliminated because there is no removal of the wax pattern from the die once it is waxed.[5] The molten gold alloy is cast directly onto the refractory die, resulting in a casting that is the exact mirror image of the die and tooth preparation. Every bur mark, undercut, and detail will be reproduced on the casting due to the micro-fine particle size of the investment. Ultimately, when this technique is utilized properly, a very predictable, consistent, and precise casting will be obtained. This will save chair-side time, reduce remakes and operator anxiety, and increase both the quality of treatment and patient satisfaction. In addition, the long-term prognosis of the restoration is enhanced due to the precise fit and marginal adaptation of these restorations.[16]

This technique has been successfully employed in several Tucker Study Club sessions, during which various configurations of cast gold restorations are prepared, fabricated on-site, and delivered over a three-day period. The technique has demonstrated the potential to enable operators of varying experience to efficiently produce and deliver extremely precise, conservative restorations with a very long functional prognosis (Figure 26).

Table 2: *Materials and Equipment*

Product	Manufacturer
Aquasil Ultra Heavy Type 2 Heavy Body PVS impression material Aquasil Ultra XLV Light Body PVS impression material	Dentsply Caulk York, PA, USA www.dentsply.com
Harvest Dental MS3 Master Model Separator and Surfactant	Harvest Dental Brea, CA, USA www.harvestdental.com
Starvest Phosphate Bonded Investment (High-Expansion Green Liquid/Powder)	Emdin International Corporation Irwindale, CA, USA www.emdin.com
Investpres Pneumatic Pressurized Curing Unit	Lang Dental Manufacturing Co, Inc Wheeling, IL, USA www.langdental.com
Monotrac V2 Quadrant Articulator	Monotrac Articulation Midvale, UT, USA www.monotrac.com
Diamond Separating Disc	Brasseler USA Savannah, GA, USA www.Brasselerusadental.com
Dynex Glass-Reinforced Separating Discs (40 × 0.5 mm)	Renfert Hilzingen, Germany www.renfert.com
XTC Xtreme Temperature Coatings High-Temperature Automotive Paint	KBS Coatings Valparaiso, IN, USA www.KBS-Coatings.com
Consequent Orange Wax Grey Thowax (sculpturing wax)	Yeti Dental Engen, Germany www.yeti-dental.com
DPT Carver	Hu-Friedy, Inc Chicago, IL, USA https://www.hu-friedy.com
COE CheckBite Trays GC Fujirock Golden Tan Die Stone	GC America Alsip, IL, USA www.gcamerica.com
Dura-White Stones (CN1 HP)	Shofu Dental Corporation San Marcos, CA, USA www.shofu.com
Elite HD soft putty	Zhermack SpA Badia Polesine, Italy www.zhermack.com
Practicum 200-Gram Digital Analytical Balance	Sartorius Göttingen, Germany www.sartorius.us
Whipmix/Jelrus Infinity L30 Digital Burnout Furnace Whipmix VPM Digital Vacuum Mixer Whipmix 60-Gram Ringless Formers and Bases	Whip Mix Corporation Louisville, KY, USA www.whipmix.com

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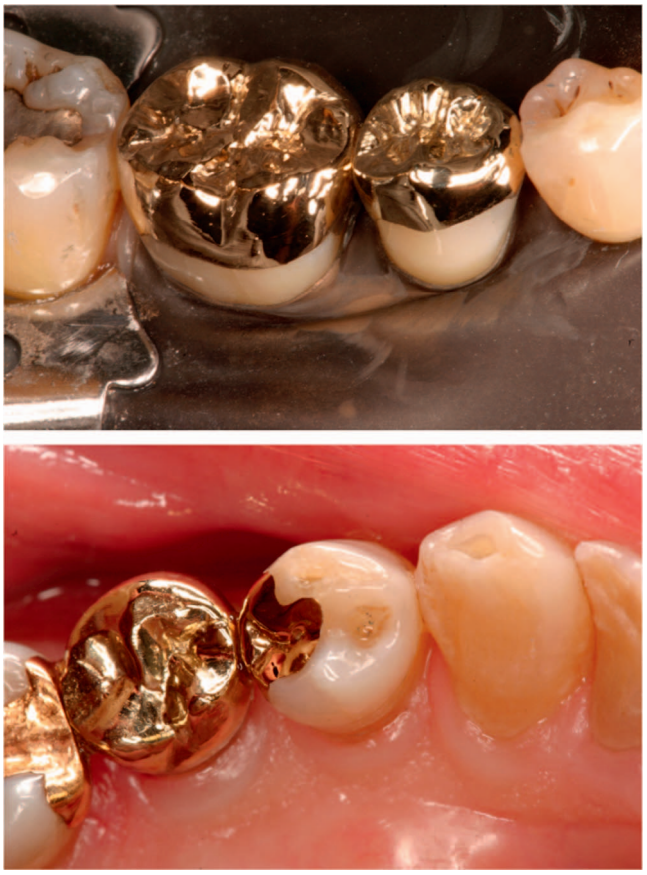

Figure 26. *Additional examples.*

Conflict of Interest

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

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Ceramic Veneers for Esthetic Restoration of Retained Primary Teeth: A 4-year Follow-up Case Report

MS Bin-Shuwaish

Clinical Relevance

The clinical longevity of esthetic ceramic restorations bonded to retained primary teeth is not well documented. This case report provides clinical evidence of the 4-year survival of ceramic veneers used for the esthetic rehabilitation of retained primary canines with mild teeth malalignment.

SUMMARY

Retained primary teeth in the smile zone can cause patient dissatisfaction, especially if associated with malposition of teeth. Orthodontic and prosthetic treatment options to treat the situation may not be accepted by some patients. Therefore, these patients tend to maintain their primary teeth and seek different esthetic options. Ceramic veneers may provide an esthetic treatment option to restore these teeth. However, the survival rates of ceramic restorations in such cases have not yet been established. This case report provides a step-by-step clinical description of the use of ceramic veneers for the restoration of retained primary canines and mild teeth malalignment, with a 4-year follow-up report, in a 28-year-old female patient. During restorative treatment, an effort was made to maintain the occlusion in group function to minimize stress and eliminate destructive forces on the retained primary teeth. After 4 years of function, the patient was still satisfied with the provided treatment.

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INTRODUCTION

Interest in achieving a beautiful smile with bright and well-aligned teeth is dramatically increasing worldwide. Patient dissatisfaction with the appearance of their teeth, and factors related thereto, has been investigated.[1-4] Tin-Oo and others reported dental appearance dissatisfaction in about 53% of 235 adult patients; and of these, around 32% blamed poorly aligned teeth.[3]

Retained primary teeth in the smile zone may cause patient dissatisfaction because of the size, alignment and/or color, along with associated malalignment of other teeth.

Persistent primary teeth are usually associated with impaction or agenesis of their successors. The prevalence of impacted permanent maxillary canines in adult dentition has been well-documented and varies according to population; it is estimated to be between 1% and 3.3% in different parts of the world.[5-10]

Patients with impacted maxillary canines must undergo clinical and radiographic evaluation before treatment. The usual orthodontic treatment of such cases involves surgical exposure of the impacted tooth, then guided orthodontic traction to align the tooth into the dental arch after creation of an adequate space following the extraction of the retained primary tooth.[11]

Multiple complications are associated with this treatment, including bone loss and injury to the adjacent teeth and soft tissue around the treated teeth.[12-14] Therefore, maintaining retained sound primary teeth may be justified. The literature supports keeping healthy retained primary teeth in adults when such teeth are nonmobile and functioning.[15,16]

It has been stated in the literature that if the tooth structures of retained primary teeth are in good condition, but in poor occlusion or otherwise requiring esthetic improvement, the primary tooth may be retained and reshaped with suitable direct or indirect restorations, such as composite or porcelain.[15] To reshape, resize, or minimally realign teeth, dentists' choices include: composite resin or ceramic laminate veneers.

Direct and indirect composite veneers can be used to address cosmetic dental problems by closing diastemas, changing tooth color, and/or resizing the teeth. However, composite restorations have certain limitations in color stability, wear resistance, and polymerization behavior.[17]

Direct composites are conservative restorations. They are easy to repair, have a relatively low cost, and achieve acceptable esthetic results; therefore, they are indicated in young patients.[18,19]

Indirect composite veneers have been shown to have properties superior to those of the direct composite restorations.[20,21] However, the quality of veneer surfaces, marginal staining, and patient satisfaction were found to be better in ceramic veneers compared with bonded indirect composite restorations.[22,23]

Ceramic laminate veneers are considered to be among the most popular esthetic restorations in clinical dentistry today. The wide acceptance of such esthetic restorations among patients is attributable to the use of ceramic veneers to solve dental problems like minimal malalignment, spaces, tooth discoloration, and/or defects.

The prognosis for any treatment option is critical to the success of the planned treatment. The correct management of retained primary teeth can extend the prognosis for their maintenance. Studies have shown that the prognosis for maxillary and mandibular primary canines is better than that for incisors and first molars.[24,25]

Only a few clinical cases on the restoration of retained primary teeth with ceramic veneers have been reported.[26-31] However, the survival rates of ceramic restorations in such cases have not yet been established. Therefore, this case report describes the esthetic rehabilitation of retained primary canines with malaligned maxillary anterior teeth by using ceramic laminate veneers; a 4-year follow-up is also presented.

CLINICAL CASE REPORT

A 28-year-old medically fit female patient presented to the restorative dentistry clinic seeking an esthetic restorative treatment for her malaligned maxillary teeth in order to achieve a more attractive smile. The patient summarized her chief complaints (in the vernacular) as follows: 1) crooked upper front teeth, 2) remaining unattractive baby teeth, and 3) long upper two centrals.

In a review of her dental history, the patient mentioned that she had performed home bleaching about 2 weeks previously and had no previous history of clenching or grinding her teeth or any other parafunctional habits. She also mentioned a prior orthodontic consultation but preferred to seek different options, such as ceramic veneers.

Clinical Examination

Oral examination revealed the following (Figure 1a through d):

- Good oral hygiene
- Right and left Class I malocclusion with maxillary and mandibular dental crowding
- Retained maxillary right and left primary canines (teeth numbers C and H)

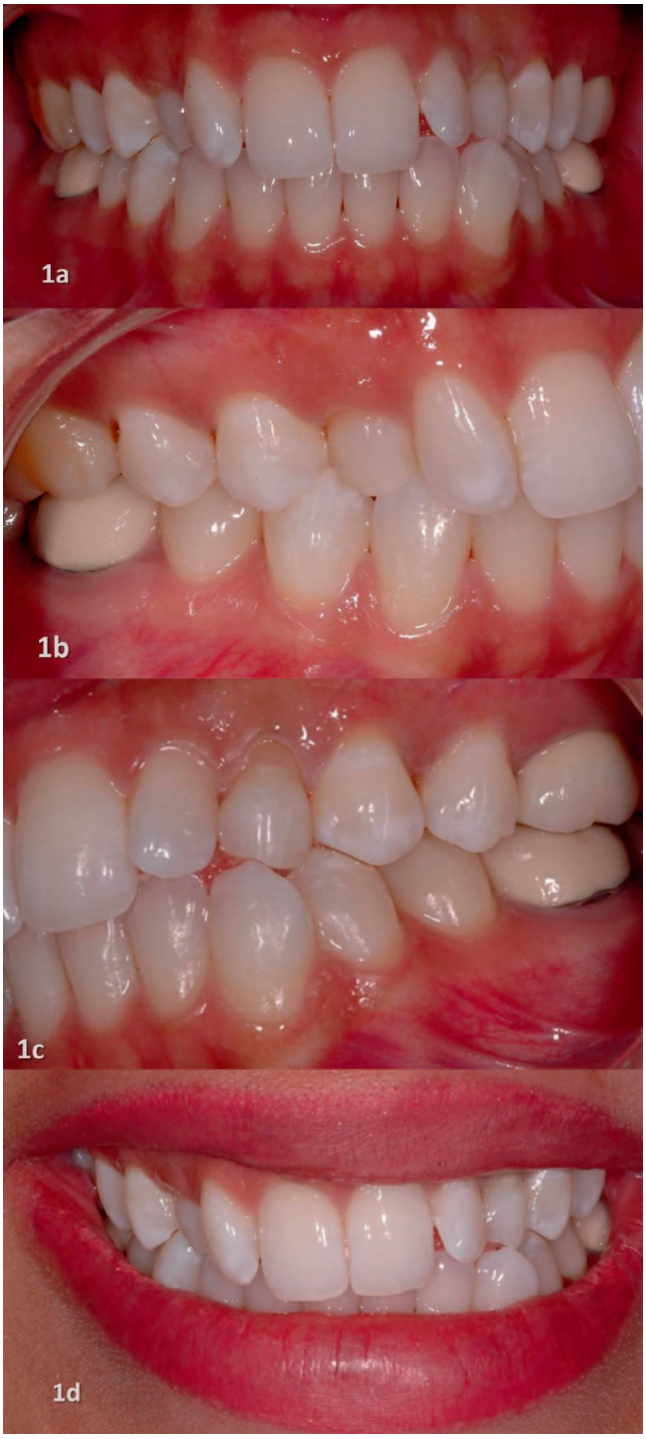

Figure 1. *Preoperative images showing teeth malalignment with retained primary maxillary canines. (a): Front view. (b): Right lateral view. (c): Left lateral view. (d) Front smile view.*

- Missing maxillary right and left permanent canines (teeth numbers 6 and 11)
- Rotated maxillary right and left lateral incisors and mandibular left canine (teeth numbers 7, 10, and 22)
- No mobility on the retained primary canines
- No periodontal pockets

Radiographic examination revealed the presence of impacted maxillary permanent canines and retained primary predecessors with good roots.

Treatment Options

Although the patient was not interested in orthodontic treatment, both orthodontic and oral surgery consultations were performed to finalize the treatment options to address her dental needs and concerns. The findings of clinical and radiographic examinations were presented and discussed with the patient, and treatment options, with potential risks and complications of each option, were presented. However, further diagnostic examinations and images were required to determine the exact locations of the impacted teeth and to evaluate the risks and time needed for the oral surgery/orthodontic treatments. Two treatment options were presented: option 1 was extraction of the retained primary canines with or without surgical extraction of impacted successor teeth, orthodontic treatment to correct the malalignment, and then implants to replace the maxillary permanent canines; option 2 was extraction of the retained primary canines, followed by surgical exposure and guided traction of the impacted permanent canines, and orthodontic treatment to correct the malalignment.

However, the patient wanted to maintain her primary teeth for as long as possible and refused any surgical treatment to expose or extract the impacted canines. She was interested in achieving esthetic results with less-invasive procedures, less time and without orthodontia. Therefore, the following options were presented, with their limitations and disadvantages: option 3 was maintaining the retained primary canines and restoring them with composite resin restorations; and option 4 was maintaining the retained primary canines and preparing them, with the maxillary anterior teeth, for ceramic laminate veneers.

After thorough discussion, the patient opted to veneer eight maxillary teeth, from the maxillary right first premolar to maxillary left first premolar, including the retained primary canines, with ceramic laminate restorations. However, she was informed that the impacted permanent canines would need to be monitored for early diagnosis of bony lesions or resorption.

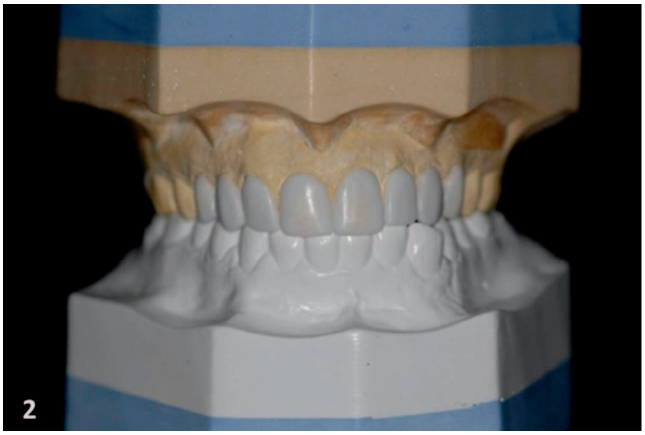

Figure 2. *Front view of the diagnostic wax-up for teeth 5, C, 7, 8, 9, 10, H, and 12, which were planned to receive ceramic laminate veneers.*

Diagnostic Wax-up

Maxillary and mandibular alginate impressions (Jeltrate, Dentsply Pharmaceuticals, York, PA, USA) were taken for the construction of study models and fabrication of maxillary diagnostic wax-ups for the teeth that were planned for ceramic laminate veneers. These were assessed, visualized, and discussed with the patient, along with the possible outcomes of the proposed treatment (Figure 2). The diagnostic wax-up model was duplicated for construction of a custom transparent plastic stent to be used in the fabrication of the temporary veneers.

Tooth Preparation and Temporary Veneers

Tooth preparation was initiated with nos. 834-016 and 834-021 depth-oriented diamond burs of 0.3 mm and 0.5 mm diameter, respectively (Hager & Meisinger GmbH, Neuss, Germany). Diamond burs nos. 852-012 and 852-014 (Hager & Meisinger GmbH) were then used to prepare the teeth, with reduction ranging from 0.3 mm to 0.5 mm, with equal gingival or slightly supragingival chamfer finish lines. The incisal edges were reduced for about 1.0 to 1.5 mm with round facial incisal line angles and a butt-joint margin along the lingual incisal edges. For teeth numbers 5 and 12, facial round chamfer preparations were used without reduction in the lengths of the cusp edges to preserve the natural occlusal surface. The interproximal contacts were left intact for all teeth. For lateral incisors, more reduction was performed, especially on tooth number 10, to correct the malalignment of these teeth. To preserve the structure of the retained primary canines, the facial surface of tooth number C received minimal facial reduction, while tooth number H was just rounded, with about 0.3 mm chamfer finish line on both teeth.

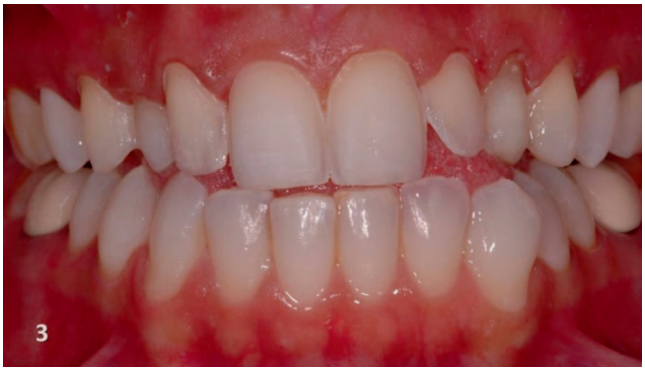

Figure 3. *Front view of the prepared teeth 5, C, 7, 8, 9, 10, H, and 12 for ceramic laminate veneers.*

After the preparation was finalized (Figure 3), Ultrapak size 0 retraction cord (Ultradent, South Jordan, UT, USA) was placed around each tooth. An immediate dentin sealing procedure was carefully performed on the areas of exposed dentin on teeth numbers 7 and 10 using OptiBond Solo Plus adhesive bonding agent (Kerr, Orange, CA, USA) according to the manufacturer's instructions. The final impression was taken with President Plus polyvinyl siloxane impression material (Coltène Whaledent AG, Altstätten, Switzerland) in a full-arch tray, and bite was registered with a face bow record.

After discussion with the patient, A1 Vitapan classic shade was selected as a stump-shade for the prepared teeth (VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany), and BL2 bleach shade (Ivoclar Vivadent AG, Schaan, Liechtenstein) was the selected shade for the final laminate veneers.

Temporary veneers were fabricated with self-cure provisional material Systemp.c&b II (Ivoclar Vivadent AG) in a customized plastic stent. The spot-etch technique was used to help hold the temporary in place by applying a very small drop of Total Etch 37% phosphoric acid (Ivoclar Vivadent AG) onto the facial surfaces of prepared teeth for 10 seconds, after which teeth were thoroughly rinsed for 30 seconds and air-dried with gentle air spray. Areas of preparation that were treated with immediate dentin sealing were isolated with a separating layer of petroleum jelly before fabricating the temporary veneers. Interproximal embrasures of the splinted provisional restoration were opened, and gingival excess was removed carefully by means of a Bard-Parker knife with scalpel blade number 12. Occlu-

sion was then checked, and the provisional was finished and polished.

The final impression with the mandibular cast model and the bite registration were sent to the dental laboratory, where ceramic laminate veneers were fabricated with the BL2 shade of the IPS e.max Press lithium disilicate glass-ceramic system (Ivoclar Vivadent AG). Instructions were given for maintaining the occlusion in a bilateral group function with decreased load on the retained primary canines to protect them during function.

Cementation

Before the patient arrived for the cementation visit, the ceramic veneers were tried-in on the working cast to evaluate the contours, marginal fit, proximal contacts, and axial contours.

The provisional veneers were carefully removed, and teeth were cleaned with a soft rubber cup and fine pumice. Teeth were isolated with cotton rolls, and laminate veneers were tried-in individually and all together in the presence of the transparent shade of the Variolink II Try-In paste (Ivoclar Vivadent AG).

After careful evaluation and patient's approval of the laminate veneers, the tissue surface of each veneer was prepared for final cementation. Cleaning with Total Etch 37% phosphoric acid (Ivoclar Vivadent AG) was performed followed by thorough rinsing. Each veneer was acid-etched according to the manufacturer's instructions with 9% hydrofluoric acid for 20 seconds (Ultradent), thoroughly rinsed with water spray, and dried with oil-free air.

A thin layer of Monobond-S silane primer (Ivoclar Vivadent AG) was then applied to the tissue surface and left for 60 seconds. Subsequently, any remaining excess was dispersed with a stream of air.

Thin, clear Mylar matrix strips (Patterson Dental Supplies, St Paul, MN, USA) were used to protect adjacent tooth structures from the etchant/bonding agents. Prepared tooth surfaces were acid-etched for 15-30 seconds with Total Etch 37% phosphoric acid (Ivoclar Vivadent AG), thoroughly rinsed with water/air spray for 30 seconds, then lightly air-dried with a gentle stream of compressed air. Adhesive bonding agent (Excite DSC, Ivoclar Vivadent AG) was then applied, according to the manufacturer's instructions, on the prepared tooth surfaces for 10 seconds, followed by light drying with a gentle stream of compressed air to spread an even thickness of the bonding agent on the prepared tooth surfaces and to evaporate the solvent in the bonding agent. The bonding agent was then light-cured for 10 seconds with an Elipar S10 LED curing unit at 1100 mW/cm^2 (3M ESPE, Minneapolis, MN, USA).

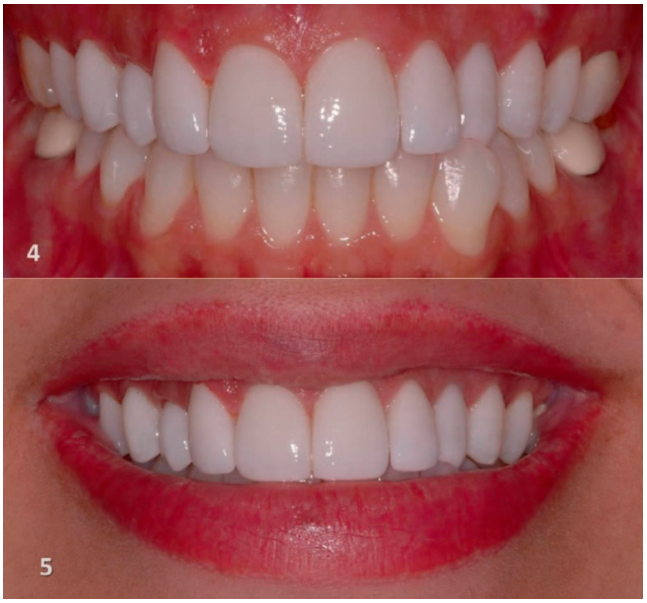

Figure 4. *Front view of the cemented ceramic laminate veneers.*
Figure 5. *Postoperative front view of the patient's smile.*

Laminate veneers were cemented, starting with the two central incisors, with a transparent shade of Variolink II base resin cement (Ivoclar Vivadent AG) according to the manufacturer's instructions. Veneers were light-cured for 40 seconds on the gingival, facial, incisal, mesial, and distal surfaces of each veneer. The excess luting cement was gently removed, and occlusion was examined in centric and eccentric movements by means of ultrathin articulating paper (Henry Schein, Melville, NY, USA) to ensure that the occlusion was maintained in a bilateral group function and to eliminate contacts on the retained primary teeth during eccentric movements. Occlusion was adjusted with fine finishing diamond burs then polished with diamond-impregnated cups and points (Brasseler, Savannah, GA, USA). Veneers were then viewed from each profile and were finally inspected by the patient (Figures 4 and 5).

After 1 week, the patient returned for evaluation of her gingival health and any further adjustment or finishing. She was satisfied with the result of the cemented laminate veneers and reported no sensitivity or discomfort.

Four-Year Follow-up

A 4-year follow-up visit was arranged to evaluate the status of the retained primary teeth, the

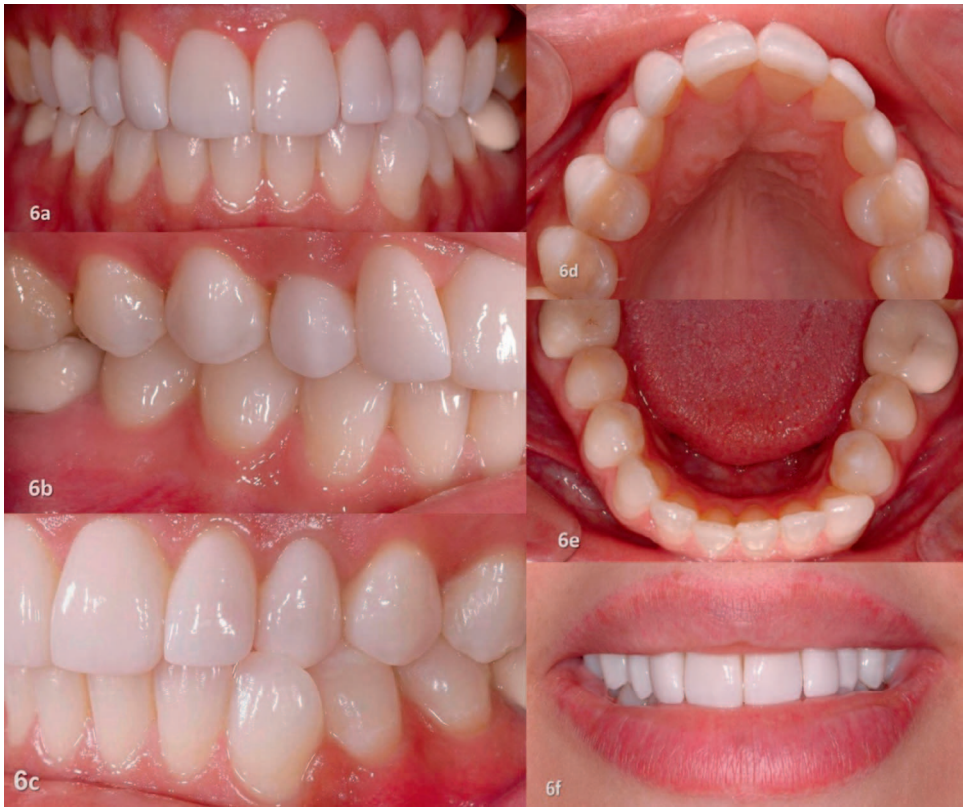

Figure 6. *Four-year follow-up images. (a): Front view. (b): Right lateral view. (c): Left lateral view. (d): Maxillary occlusal view. (e): Mandibular occlusal view. (f); Front smile view.*

occlusion and the ceramic laminate veneers (Figure 6a through f). No mobility, caries lesions or periodontal pockets were found on the retained primary teeth. No marginal gap, fracture, or debonding was observed on the laminate veneers. There was no sensitivity or discomfort on the veneered teeth. Some plaque accumulation, mild gingival inflammation, and staining could be seen on some of the teeth. Therefore, oral hygiene instructions were reinforced, and the patient was referred to the hygienist for professional tooth cleaning. Radiographs showed no noticeable changes in the status of the retained primary teeth or the impacted permanent teeth.

DISCUSSION

Dental malalignment associated with small-sized retained primary teeth may dramatically affect the esthetics of the anterior teeth and, therefore, the smile. This case report describes a detailed procedure for esthetic rehabilitation of the maxillary anterior teeth in a 28-year-old female patient with retained primary canines and mild dental malalignment who presented to the dental clinic to improve her smile. A list of the materials and instruments used in this case is shown in Table 1.

Treatment of impacted maxillary canines is considered a common clinical challenge.[32] Comprehensive diagnostic evaluation is required to finalize the treatment options. Options of treatment may include[6,33] 1) oral surgery and orthodontic and prosthodontic treatments, or 2) no treatment if the patient does not accept the first option or if the impacted canines are severely displaced with no evidence of adjacent pathology or teeth resorption. However, a periodic clinical and radiographic evaluation should be maintained so that any pathologic changes can be noted.

Surgical intervention with orthodontic and prosthetic treatments may provide a good long-term prognosis with reliable results, especially for young patients; therefore, this is a commonly accepted treatment option. However, this option may not be accepted by others because of the lengthy procedures and risks involved, including possible surgical complications, bone loss, and/or periodontal problems.[12-14] Therefore, these patients tend to seek different treatment options.

Advantages and Limitations

In this case, restorative treatment options with ceramic or composite veneers were offered to the

Table 1: *List of Materials and Instruments Used in This Case*

Material/Instrument	Description/Use	Manufacturer
Jeltrate	Alginate impression material	Dentsply Pharmaceuticals, York, PA, USA
834-016 bur	Depth-oriented diamond burs	Hager & Meisinger GmbH, Neuss, Germany
834-021 bur		
852-012 bur	Tapered round-end chamfer diamond burs	Hager & Meisinger GmbH.,Neuss, Germany
852-014 bur		
Ultrapak	Tissue retraction cord	Ultradent, South Jordan, UT, USA
Total-Etch	37% phosphoric acid etchant	Ivoclar Vivadent AG, Schaan, Liechtenstein
OptiBond Solo Plus	Adhesive agent (for immediate dentin sealing)	Kerr, Orange, CA, USA
President Plus	Polyvinyl siloxane impression material	Coltène Whaledent AG, Altstätten, Switzerland
A1, VITAPAN	Classic shade tab	VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany
BL2	Bleach shade tab	Ivoclar Vivadent AG, Schaan, Liechtenstein
Systemp c&b II	Self-cure provisional material	Ivoclar Vivadent AG, Schaan, Liechtenstein
12-fluted carbide and fine diamond burs	Finishing of provisional restorations	Ultradent, South Jordan, UT, USA
Rubber points and cups	Composite polishing kit	Shofu Inc, Kyoto, Japan
IPS e.max Press	Lithium disilicate glass-ceramic veneers	Ivoclar Vivadent AG, Schaan, Liechtenstein
Variolink II Try-In	Try-in paste	Ivoclar Vivadent AG, Schaan, Liechtenstein
Ultradent Porcelain Etch	9% hydrofluoric acid	Ultradent, South Jordan, UT, USA
Monobond-S	Silane primer	Ivoclar Vivadent AG, Schaan, Liechtenstein
Mylar strip	A clear matrix band	Patterson Dental Supplies, St Paul, MN, USA
Excite DSC	Adhesive bonding agent	Ivoclar Vivadent AG, Schaan, Liechtenstein
Elipar S10 LED	Light-curing unit	3M ESPE, Minneapolis, MN, USA
Variolink II	Resin luting cement	Ivoclar Vivadent AG, Schaan, Liechtenstein
OptraStick	Veneer placement tip	Ivoclar Vivadent AG, Schaan, Liechtenstein
Articulating Papers	Occlusion checker	Henry Schein, Melville, NY, USA
Fine diamond burs and diamond-impregnated cups and points	Porcelain finishing and polishing kits	Brasseler, Savannah, GA, USA

patient. Advantages, disadvantages, and limitations of the composite resin restorations, such as color instability, limited mechanical properties, and overall esthetic results were explained.

Advantages of ceramic veneers, including the ability to recreate natural tooth dimensions and proportions, correct malalignment, and change tooth color were also discussed. Conversely, the disadvantages of tooth preparation for ceramic laminate veneers and limitations of their repairability compared with those of composite resin, in addition to the limitations to achieve good alignment were all explained. The patient was also informed that tooth number 10 would need more aggressive reduction to create acceptable alignment.

Potential Problems

In the present case, after an orthodontic and oral surgery consultation, the patient decided to keep and modify her primary canines without surgical or orthodontic intervention. The risks of root resorption, extensive caries, and/or periodontal disease, all of which can lead to extraction of the retained primary teeth, were explained. The patient was also informed about the prosthetic options for replacing these teeth.

Dentin Exposure and Postoperative Sensitivity

Teeth with malalignment may require aggressive reduction, with care taken to avoid pulpal damage during tooth preparation. In the present case, both lateral incisors required more reduction (especially tooth number 10); therefore, an effort was made to align these teeth as much as possible without violating the pulp.

Exposing dentin during the preparation of malaligned teeth or teeth with gingival recession is inevitable. Hence, immediate dentin sealing may be used to improve dentin bond strength and decrease postoperative sensitivity by the gentle application of a dentin bonding agent to the freshly cut dentin surfaces immediately after tooth preparation.[34-38] A

light-cured three-step total-etch bonding agent was used here.

Long-Term Prognosis

The long-term prognosis of restored retained primary teeth depends on multiple factors, such as root resorption and risk of destructive recurrent caries, periodontal disease, and debonding or failure of the restoration. In this case, an effort was made to maintain occlusion in a bilateral group function to minimize stress and eliminate destructive forces on the retained primary teeth during function. The rate of root resorption of the retained primary teeth varies widely among individuals and has been found to decrease with age.[39]

Bond strength to the tooth structure is an important factor in the survival of the bonded restoration. Optimum bond strength of resin restorations to the conditioned enamel and dentin of primary teeth has been documented in the literature.[40-42] Furthermore, this bond strength was found to be similar to that of the permanent teeth.[43-47] Meola and Papaccio[48] reported that grinding the enamel surface with a diamond bur helps remove the prismless enamel layer, resulting in improved bond of the restoration to the enamel.

Survival of ceramic veneers has been well documented in the literature.[49-52] A recent investigation evaluated the 21-year cumulative survival rate of porcelain laminate veneers bonded to prepared enamel and estimated it to be 96% ± 2%.[49]

In the present case, a lithium disilicate glass-ceramic system (IPS e.max Press, Ivoclar Vivadent AG) was used to fabricate the laminate veneers. The fracture rate of IPS e.max ceramic veneers in a 4-year cross-sectional study was found to be relatively low (1.3%).[52]

In another study, lithium disilicate ceramic veneers cemented on 60 incisors and canines with different preparation designs showed 100% survival rates.[51] Although it has been reported in the literature that retained primary canines have a good predictable life span,[24] the long-term prognosis of the restored retained primary teeth remains unclear. Therefore, further research and clinical studies in this area are warranted.[15]

CONCLUSIONS

- Using ceramic laminate veneers to restore maxillary retained primary canines can provide satisfactory and esthetic clinical results in terms of correcting mild discrepancies in tooth shape, size, and occlusion.
- This case report provides clinical evidence of an esthetic restorative treatment of malaligned maxillary anterior teeth, with retained primary canines, by the use of ceramic laminate veneers; the 4-year follow-up is also reported.
- It is critical that the patient understand the limitations of this treatment option and the odds of losing one or both retained primary teeth in the future, as well as proposed treatment options to replace them.
- More research and clinical reports are needed on the long-term survival of indirect restorations on retained primary teeth.

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Clinical Research

Influence of Adhesive Type and Placement Technique on Postoperative Sensitivity in Posterior Composite Restorations

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P Dalzochio • AD Loguercio • A Reis

Clinical Relevance

The use of a single increment of new bulk-fill material, even in deep cavities, did not generate more postoperative sensitivity when compared to its use in an incremental filling technique. Similarly, the adhesive strategy did not have any impact on postoperative sensitivity.

SUMMARY

Purpose: This double blind, randomized clinical trial compared the postoperative sensitivity of the placement technique (incremental and bulk fill) in posterior composite resin restorations bonded with two different adhesive strategies (self-etch and etch-and-rinse).

Methods: Posterior dental cavities of 72 participants (n=236), with a cavity depth of at least 3 mm, were randomly divided into four groups. The restorations were bonded using either the etch-and-rinse Tetric N-Bond (Ivoclar Vivadent) or the self-etch Tetric N-Bond SE (Ivoclar Vivadent). The composite resin Tetric N-Ceram Bulk Fill (Ivoclar Vivadent) was placed either incrementally or using the bulk-fill technique. Two experienced and calibrated examiners evaluated the restorations using World Dental Federation criteria after one week of clinical service. Spontaneous postoperative sensitivity was assessed using a 0-4 numerical rating scale and a 0-10 and 0-100

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visual analog scale up to 48 h after the restorative procedure and after one week.

Results: The risk ($p>0.49$) and intensity of spontaneous postoperative sensitivity ($p>0.38$) was not affected by the adhesive strategy or the filling technique. The overall risk of postoperative sensitivity was 20.3% (95% confidence interval 15.7-25.9) and typically occurred within 48 hours after the restorative procedure.

Conclusions: The overall risk of immediate postoperative sensitivity was 20.3% and was not affected by either the adhesive strategy (etch-and-rinse/self-etch) or the filling technique (incremental/ bulk).

INTRODUCTION

Incremental layering has long been accepted as a standard technique for placing composite resins in cavity preparations.[1] Typically, this technique consists of placing composite resin in increments with a maximum thickness of 2 mm to ensure adequate curing. This procedure produces a composite resin restoration with enhanced physical properties (when compared with chemically cured composite resins), improved marginal adaptation, and reduced cytotoxicity.[2,3] Incremental filling also reduces polymerization shrinkage by reducing the volume of composite resin placed as well as the C-factor, which is the ratio of bonded to unbonded areas of the cavity preparation.[4,5]

On the other hand, the incremental filling technique has some drawbacks.[6] Contamination or incorporation of voids between layers can occur. When compared to bulk filling, this technique is more time demanding, which is contrary to the clinician's desire for simplified and fast procedures.[7,8] These drawbacks have led some manufacturers to introduce composite resins for "bulk-filling techniques," allowing composite resin placement in increments of up to 5 mm thickness.

Contrary to the old version of bulk-fill composite resins,[9,10] these newest bulk-fill composite resins have adequate degree of conversion,[11] microhardness,[12,13] low volumetric shrinkage, and high depth of cure,[14-16] even when used at a thickness of 4 mm.

Despite these promising laboratory findings, some studies have reported that the placement of composite resins in 4- or 5-mm-thick increments may increase cuspal deflection[17,18] and the development of stress at the adhesive interface,[19] which may be clinically apparent by increased postoperative sensitivity. Other studies, however, have reported that the filling technique has no influence on polymerization shrinkage at the adhesive interface[20-22] and that the adhesive strategy may be more important than the composite resin placement technique[23] in preventing the deleterious effects of polymerization stress.

There are currently two available adhesive strategies for use with composite resins: etch-and-rinse and self-etch.[24] Etch-and-rinse adhesives use phosphoric acid for substrate conditioning before adhesive application. After phosphoric acid rinsing, dentin hydration should be adequately managed; otherwise, resin monomers cannot infiltrate into the demineralized dentin[25] and cannot seal dentin tubules,[26] increasing the chances of postoperative sensitivity. Self-etch adhesives do not require multiple steps for bonding. The simultaneous application of a primer and an acidic monomer in a single step results in a lower discrepancy between dentin demineralization and resin infiltration into the dentin,[27] which may reduce postoperative sensitivity when compared to the etch-and-rinse technique.[28,29] However, the role of adhesive strategy on postoperative sensitivity in posterior teeth is still controversial.[30,31]

Therefore, the objective of this double blind, randomized clinical trial was to compare the postoperative sensitivity of the placement technique (incremental or bulk fill) in posterior composite resin restorations bonded with two different adhesive strategies (self-etch or etch-and-rinse). The null hypotheses tested were that 1) the layering technique and 2) the adhesive strategy do not influence the postoperative sensitivity of posterior composite resin restorations.

METHODS AND MATERIALS

This study was written according to the CONSORT statement.[32] The study was conducted at the clinic of the School of Dentistry from the State University of Ponta Grossa, Brazil. All participants were informed about the nature and objectives of the study; however, they were not aware of which treatments their lesions would receive. The Ethics Committee of the State University of Ponta Grossa (Paraná, Brazil) reviewed and approved this study under protocol number 109.846. A written informed consent was obtained from all participants before starting the treatment.

Study Design

This was a double blind (evaluator and patient) and split-mouth randomized clinical trial with four study groups and an equal allocation ratio.

Participant Recruitment

Two calibrated dental residents performed the selection of the participants, using a mouth mirror, an explorer (SS White, Rio de Janeiro, Brazil), and a periodontal probe (#6 Satin Steel Handle; mm, Hu-Friedy, Chicago, IL, USA). We recruited eligible patients in the order they appeared for the screening session in the Dental Clinic at the Local University, thus forming a convenience sample of patients.

Sample Size Calculation

The risk of postoperative sensitivity was the dependent outcome used for sample size calculation (primary outcome). Earlier clinical trials reported that the risk of postoperative sensitivity in deep and wide cavities in posterior teeth was approximately 30%.[29,33,34] In order to identify a difference of 20% in the risk of postoperative sensitivity between any of the experimental conditions, a minimum sample size of 59 restorations per group were required, with an alpha of 5% and a statistical power of 80%. The sample size calculation was performed using a software program that is freely available online at http://www.sealedenvelope.com.

Inclusion and Exclusion Criteria

Selected participants had to be at least 18 years old. They had to have at least 12 posterior teeth in occlusion and not receiving orthodontic treatment. Each patient had to have at least two or four posterior teeth in need of restorative treatment (due to carious lesions, defective restorations with secondary caries, fractures, or a patient request for replacement due to esthetic reasons). The dental cavities had to be at least 3 mm deep, which was diagnosed using a bitewing radiograph and a ruler. Each tooth to be restored had to be in "normal occlusion" with natural antagonist and adjacent teeth.

According to the rules of the School of Dentistry from our university, patients are able to receive restorative treatment only when they are no longer caries active and they have reached a good level of oral hygiene. For this purpose, the patients selected for this study were instructed about oral hygiene and diet. Staining with dyes was performed weekly; patients received restorations only when low biofilm formation was observed.

We checked the pulp vitality with a cold test (Roeko-Endo-Frost, Coltène/Whaledent, Langenau, Germany). After 10 seconds of cold application, participants were expected to answer positively to this test providing a short and transient pain response. We excluded participants needing endodontic treatment or those with non-vital teeth and periodontal problems. Pregnant or breast-feeding women, participants with known allergies to resin-based materials or any other material used in this study, and those taking anti-inflammatory, analgesic, or psychotropic drugs were not included in this study.

Characteristics of the Cavities and Calibration of Operators

For each restoration, the tooth type (molar/premolar) and cavity type (number of restored surfaces) were recorded. A bitewing radiograph of each tooth was taken with an exposure time of 0.4 seconds using an X-ray device (Timex 70 E, Gnatus, Ribeirão Preto, Brazil) set at 70 kVp and 7 mA.

Four operators conducted all restorative procedures. At the time the study was conducted, all the operators had five or more years of clinical experience and all were PhD students specializing in restorative dentistry. The operators measured the height and depth of the proximal and occlusal cavity boxes with a periodontal probe (#6 Satin Steel Handle; mm, Hu-Friedy).

For the calibration of the operators, one professor, a specialist in restorative dentistry with more than 15 years of clinical and research experience, placed one restoration of each group in order to identify all restorative steps involved in the application technique. Then each operator placed four restorations of each group under the supervision of the experienced clinician. The restoration deficiencies were shown to the operators prior to starting the study and discussed. After that, the operators were considered to be calibrated and able to perform the restorative procedures. The same calibrated dental residents who participated in the patient screening selection restored all teeth under the supervision of an experienced clinician.

Randomization and Allocation Concealment Mechanism

We performed two different randomization schemes: one for the subjects with four teeth and another scheme for the subjects with two available teeth for restoration. For the subjects with four teeth, the randomization was done on an intra-individual basis so that each subject ended up with four restorations, each one resulting from one of all possible combinations of filling technique (incremental filling [IF] or

bulk filling [BF]) and adhesive strategy (etch-and-rinse or self-etch).

In the patients with two teeth, two different randomization lists were performed with block sizes of two and four (to guarantee an equal number of restorations in the groups and prevent disclosure of the allocation concealment). The first randomization list defined the type of adhesive strategy used in that patient. The second randomization list defined the order of the composite resin placement.

These randomization schemes were performed using software available at http://www.sealedenvelope.com. A researcher not involved in the any of the experimental phases performed this procedure. The randomization lists were numbered consecutively and individually placed in opaque and sealed envelopes. These envelopes were opened at the day of the restorative intervention to prevent disclosure of the randomization scheme.

In all cases, the tooth with the highest tooth number received the treatment described first, while the tooth with the next number in sequence received the treatment mentioned second, with placement continuing in a similar manner until the fourth tooth (for the patients with four teeth). All restorations in the same subject (two or four) were always placed in different sextants.

Study Blinding Protocols

The operator who implemented the interventions was not blinded to the procedure. However, participants and the evaluators were kept blind to the group allocation during examinations.

Interventions: Restorative Treatment

All patients received oral hygiene instructions and a professional tooth cleaning before initiating the restorative intervention. The operators anesthetized the teeth (Mepisv 3%, NovaDFL, Rio de Janeiro, Brazil) and performed rubber dam isolation. The cavity design was restricted to the elimination of carious tissue or defective restorations using a spherical diamond bur (#1015-1017; KG Sorensen, Barueri, Brazil) mounted in a high-speed handpiece with air-water spray. No liner or base was used. For restoration of class II cavities, a sectional matrix system (Palodent, Dentsply Caulk, Millford, DE, USA) was preferentially used. However, circumferential matrix systems were used when a good adaptation could not be obtained with the sectional matrix system.

Two adhesive systems were used: Tetric N-Bond (two-step etch-and rinse, Ivoclar Vivadent, Schaan, Liechtenstein) and Tetric N-Bond SE (one-step self-etch, Ivoclar Vivadent). The adhesives were applied following the manufacturer's instructions (Table 1). The composite resin Tetric N-Ceram Bulk Fill (also known as Tetric EvoCeram Bulk-Fill in other countries, Ivoclar Vivadent) was placed according to either the incremental or the bulk-filling techniques (Table 1).

In the groups assigned for increment filling, we restored the dental cavity with 2-mm-thick horizontal layers. In the incremental filling technique, a small increment of resin composite was removed from the compule, shaped into a ball using the right thumb and index finger, and finally placed in the cavity with a resin spatula. During this step, the operators wore clean gloves to avoid contamination between composite resin layers. In the bulk-filling groups, one 4-mm-thick horizontal layer was placed at the bottom of the cavity, as previously described in the incremental filling technique. If the cavity had a depth greater than 4 mm, additional material was added to fill the whole cavity. We also recorded the number of layers required for the restoration of each dental cavity.

The operators adapted the composite resin using a flat-faced or elliptical condenser and light cured each increment for 20 seconds using a Bluephase light-curing unit (Ivoclar Vivadent) at 1200 mW/cm^2. The curing tip was placed as close as possible to the occlusal surface of the teeth, as some light attenuation was anticipated due to the cavity depth. The light-curing output was checked daily. We performed occlusal adjustments using fine-grit diamond burs (KG Sorensen) and checked the quality of the interproximal contact and the cervical adaptation by means of dental flossing and bitewing radiographs (using the same parameters as described earlier).

We performed finishing and polishing immediately after the final light-curing step using fine-grit diamond burs (KG Sorensen), OptraPol NG (one-step silicon polishing set with diamond particles, Ivoclar Vivadent) and Astrobrush (Ivoclar Vivadent) under constant water-cooling. We used abrasive strips (3M ESPE, St Paul, MN, USA) on the proximal surfaces when necessary.

Clinical Evaluation

Two experienced and calibrated examiners who were not involved in the placement of the restorations

Table 1: *Application Mode of Each Material Used in the Study*

Material (Manufacturer) Batch Number	Composition	Application Mode
Total N-Etch (Ivoclar Vivadent) N05612	Phosphoric acid (37%), thickness agent and color pigments	Apply phosphoric acid on the prepared enamel and then flow the etchant onto the prepared dentin. The etchant was left to react on the enamel for 15 to 30 s and on the dentin for 10 to 15 s. After that, the phosphoric acid was removed with a vigorous water spray for at least 5 s. Excess moisture was removed with air gun, leaving the dentin surface with a slightly glossy wet appearance (wet-bonding).
Tetric N-Bond (Ivoclar Vivadent) N40889	Bis-GMA, urethane dimethacrylate, dimethacrylate, hydroxyethyl methacrylate, phosphonic acid acrylate, nanofillers (SiO_2), ethanol, initiators and stabilizers	After acid etching, apply a thick layer of adhesive on the enamel and dentin surfaces, using a microbrush. Brush the material gently into the dentin for 10 s. Remove excess material and the solvent by a gentle stream of air so that the adhesive completely covers the enamel and dentin without pooling. Light cure adhesive for 10 s (light intensity 1200 mW/cm^2). A shiny tooth surface prior to the application of the composite shows that all surfaces are completely covered.
Tetric N-Bond Self-Etch (Ivoclar Vivadent) R59913	Bis-acrylamide derivatives, bis-methacrylamide dihydrogenphosphate, amino acid acrylamide, hydroxyalkyl methacrylamide, water, nanofillers (SiO_2), initiators and stabilizers	No applied acid etching. Apply a thick layer of adhesive on the enamel and dentin surfaces, using a microbrush. Brush the material gently into the dentin for 30 s. Remove excess material and the solvent by a gentle stream of air so that the adhesive completely covers the enamel and dentin without pooling. Light cure adhesive for 10 s (light intensity 1200 mW/cm^2). A shiny tooth surface prior to the application of the composite shows that all surfaces are completely covered.
Tetric N-Ceram Bulk Fill (Ivoclar Vivadent) R52450	Dimethacrylates, prepolymer, barium glass filler, ytterbium trifluoride, mixed oxide, additive, initiators and stabilizers, pigments	Apply resin composite in layers of maximum 2 mm (incremental technique) or 4 mm (bulk technique) and contour/adapt the material to the cavity walls using a suitable instrument. Light cure each increment for 20 s (light intensity 1200 mW/cm^2).

performed the evaluation using the World Dental Federation criteria[35,36] after one week of clinical service.

For the evaluation of the primary outcome, participants were asked to record whether they experienced sensitivity using a 0-4 numerical rating scale (0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe) and a 0-10 and 0-100 visual analog scale (VAS). In the 0-10 VAS scale, the participants had to place a line perpendicular to a 10 mm line, with zero at one end, indicating "no sensitivity," and at 10 mm in the other end, indicating "unbearable sensitivity." In the 0-100 VAS scale, participants had to attribute one number varying from zero (no pain) to 100 (unbearable pain) that most represented the postoperative sensitivity. The patients were asked to fill in the pain scale forms 24 hours after the restorative procedure and daily up to seven days. Additionally, we also instructed them to record in a diary if the sensitivity was spontaneous or induced by mastication, air, heat, or cold stimuli.

The secondary clinical end points were restoration fracture/loss of retention, marginal caries, marginal staining, marginal adaptation, surface texture, and color match. We ranked these variables using the

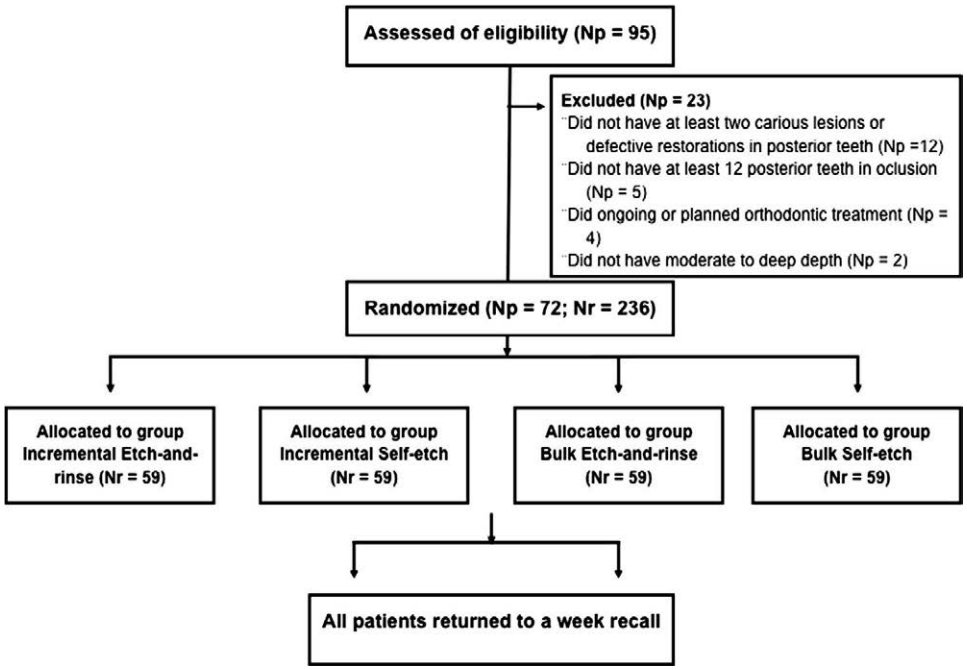

Figure 1. *Participant flow diagram in the different phases of the study design. Np, number of participants; Nr, number of restorations.*

following scores: clinically very good, clinically good, clinically sufficient/satisfactory, clinically unsatisfactory but can be repaired, and clinically poor and needing replacement. For marginal adaptation and marginal staining, we employed the semiquantitative SQUACE criterion, proposed by Hickel and others[35,36] The results of these evaluation criteria will be published at a later time.

Statistical Analysis

The statistician was blinded to the type of study groups, and the analyses followed the intention-to-treat protocol.[32] Participants who experienced at least one event of postoperative sensitivity (regardless of the intensity) during the one-week evaluation were considered as having postoperative sensitivity.

The risk of postoperative sensitivity among the groups was compared using the chi-square test and Fisher exact test. The intensity of the postoperative sensitivity was evaluated using the Kruskall-Wallis and Mann-Whitney tests. We also compared the immediate postoperative sensitivity (up to 48 hours after the restorative procedure) and one-week postoperative sensitivity (one week after the end of the procedure) with the Fisher exact test. Additionally, the risk of postoperative sensitivity according to tooth type, cavity depth, and number of faces was compared using the chi-square test. In all statistical tests, the alpha was set at 5% (Statistica for Windows 7.0, StatSoft Inc, Tulsa, OK, USA).

RESULTS

Characteristics of the Participants and Cavities

The experimental protocols were implemented exactly as planned, and no modifications were performed. Figure 1 depicts the participant flow diagram at the different phases of the study design. A total of 52 women and 20 men participated in this study. The mean age of the participants was 34 $\pm$ 10 years. Two hundred thirty-six restorations were placed, 59 for each group.

The distribution of the restorations was similar between class I (126) and class II (110) cavities (Table 2). The homogeneity of cavity characteristics between the study groups can be seen in Table 2. All of the participants attended the one-week recall.

Postoperative Sensitivity

None of the subjects needed an analgesic drug to reduce postoperative sensitivity. Only two subjects reported with postoperative sensitivity who also had preoperative sensitivity. Regardless of the group, most of the postoperative sensitivity complaints occurred within the first 48 hours after treatment.

Neither the restorative technique (incremental vs bulk) nor the adhesive strategy (etch-and-rinse vs self-etch) affected the risk (Table 3; $p>0.49$). The overall risk of postoperative sensitivity was 20.3% (95% CI 15.7-25.9). Seventy five percent (36/48) of

Table 2: *Characteristics of the Dental Arches and Cavities*

Characteristic	Number of Restorations			
	ER-IF	ER-BF	SE-IF	SE-BF
Tooth distribution				
Premolar	19	18	23	18
Molar	40	41	36	41
Spontaneous preoperative sensitivity				
Yes	9	5	9	9
No	50	54	50	50
Cavity depth				
3 mm	43	42	43	36
4 mm	8	9	9	11
>4 mm	8	8	7	12
Black classification				
I	33	32	32	29
II	26	27	37	30
Number of restored surfaces				
1	26	22	21	23
2	27	30	30	30
3	5	4	6	5
4	1	3	2	1
Reasons for replacement				
Marginal fracture	6	5	7	5
Esthetic reasons	33	32	25	32
Marginal discoloration	4	5	3	5
Bulk fracture	7	4	5	5
Primary or secondary caries lesion	9	13	19	12

Abbreviations: ER, etch-and-rinse; SE, self-etch; IF, incremental filling; BF, bulk filling.

the teeth with postoperative sensitivity occurred in only 11 patients (15% of total number of patients).

One week after treatment, the risk of postoperative sensitivity was statistically lower than the risk recorded in the periods immediately after restorative treatment (Table 4; $p<0.002$). Only 2.5% of the participants (95% confidence interval [CI] 1.2-5.4) reported spontaneous postoperative sensitivity after one week (Table 4).

Also, neither the restorative technique (incremental vs bulk) nor the adhesive strategy (etch-and-rinse vs self-etch) affected the intensity of spontaneous postoperative sensitivity (Table 5; $p>0.38$; for all pain scales).

In 18 out of 48 sensitive restorations, participants reported that the teeth were sensitive only to air. In another 10 restorations, participants reported that their teeth were sensitive only to cold. In six restorations, patients reported that their teeth were sensitive only to mastication. Some patients reported

Table 3: *Comparison of the Number of Patients Who Experienced Spontaneous Postoperative Sensitivity During the One-Week Follow-Up As Well As the Absolute Risk (n=59/Group)*[a]

Adhesive Strategy	Placement Technique	Tooth Sensitivity (Number of Patients)		Absolute Risk (95% Confidence Interval)
		Yes	No	
Etch-and-rinse	Incremental	13	46	22.0 (13-34)
	Bulk	10	49	17.0 (9-28)
Self-etch	Incremental	14	45	23.7 (15-36)
	Bulk	11	48	18.6 (12-44)
Overall		48	188	20.3 (15.7-25.9)

[a] *Chi-square test ($p>0.49$).*

that their teeth were sensitive to both air and cold (six participants), air and mastication (four participants), and cold and mastication (four participants).

When the characteristics of tooth type and cavities were evaluated, only the number of surfaces was statistically significant (Table 6; $p=0.01$). Cavities with three or four surfaces showed more postoperative sensitivity when compared with cavities that had one or two surfaces. The tooth type and cavity depth were not statistically different (Table 6; $p>0.06$).

DISCUSSION

In the present randomized clinical trial, restorations placed using the bulk-fill technique showed a risk and intensity of postoperative sensitivity similar to composite resin restorations placed with the traditional 2 mm incremental technique, and therefore we could not reject the first null hypothesis. Taking into account that one characteristic of an ideal dental composite resin restorative would be that it can be effectively cured in a single increment, facilitating placement, this is a very interesting result and may be attributed to some singular properties of the bulk-fill material used in this current study,[37] which makes it very similar to conventional composite resins cured incrementally, except for the fact that they can achieve a higher depth of cure.[12,38-40]

The material used in this study (Tetric N-Ceram Bulk Fill) was also found to have an increased translucency,[14,41] which can play a role on its good depth of cure[12,38-40] by reducing light scattering and improving deeper blue-light penetration.[42,43] However, this material also possesses a newly patented initiator system. In addition to the traditional camphorquinone/amine initiator system, Tetric N-Ceram Bulk Fill has an "initiator booster" (Ivocerin),

Table 4: *Number of Patients (%) Who Experienced Spontaneous Postoperative Sensitivity in Two Different Time Assessments (n=59/Group)*[a]

Time Assessment	Etch-and-Rinse		Self-Etch		Overall Risk (95% Confidence Interval)
	Incremental	Bulk	Bulk	Incremental	
Up to 48 h	13 (22.0%) A	10 (17.0%) A	11 (18.6%) A	14 (23.7%) A	20.3 (15.7-25.9)
One week later	2 (3.4%) B	1 (1.7%) B	1 (1.7%) B	2 (3.4%) B	2.5 (1.2-5.4)

[a] *The same letter indicates no statistically significant difference between groups (Fisher exact test,* $p<0.002$).

which is able to polymerize the material to a greater depth. Ivocerin is described as a germanium-based initiator system with a higher photocuring activity than camphorquinone/amine system because of its higher absorption in the wavelength region between 400 and 450 nm and the ability to form at least two free radicals to initiate the radical polymerization.[41]

Additionally, Tetric N-Ceram Bulk-Fill polymerized in a single increment has polymerization shrinkage values[38-40,44,45] and shrinkage stress[40,44,45] closer to the conventional composite resins cured in increments. The increased filler content can, to a certain extent, reduce the polymerization shrinkage by increasing the filler-to-monomer ratio,[5,46] but the presence of pre-polymerized particles can also contribute to the lower polymerization shrinkage of this material.[47] This also explains why the marginal gap formation and marginal integrity of bulk-fill composite resins was not statistically different than what was observed for incremental composite resins.[38,48] This result was also recently confirmed by Heintze and others.[49] In an *in vitro* study, those authors evaluated the marginal quality of composite resin restorations placed with Tetric N-Ceram Bulk-Fill (four mm) or with Tetric EvoCeram in three increments in class II cavities in molars. Microscopic evaluation showed no significant differences in marginal defects of the proximal margins when the bulk or incremental techniques were compared.[49]

In summary, *in vitro* studies have demonstrated that the Tetric N-Ceram Bulk-Fill has a good depth of cure without generating significant polymerization shrinkage and the associated residual stresses generated from shrinkage when compared to the same resin composite used with an incremental technique, explaining why we observed similar risks of postoperative sensitivity in the present study for both placement techniques.

So far, only a few clinical trials evaluated the postoperative sensitivity of bulk-fill materials in posterior restorations.[50-54] One limitation of these earlier studies is that they do not report the depth of the included cavities, which is of paramount importance when evaluating the performance of bulk-fill composite resins. Van Dijken and Pallesen[50,51] compared the bulk-filling technique (using flowable composite resin plus a capping layer made of composite resin applied in an incremental technique) with a conventional composite resin placed incrementally. Similar to the present study, these earlier studies[50,51] reported no significant differences in postoperative sensitivity between the two techniques evaluated. In another clinical study,[52-54] a single bulk-fill increment was compared to an incrementally filled composite resin in posterior restorations. However, postoperative sensitivity was evaluated only after 14 days, preventing us from comparing it with this study's findings.[52-54]

The overall absolute risk of postoperative sensitivity of the present study was higher than that observed in a recent systematic review of class II composite restorations.[30] However, in this review, the data from restorations of different studies were pooled together, and the effect of cavity depth was not taken into consideration. There are only few

Table 5: *Means (Standard Deviations) of Spontaneous Postoperative Sensitivity Experienced by Patients for All Groups Using Three Pain Scales*[a]

Pain Scales	Etch-and-Rinse		Self-Etch	
	Incremental	Bulk	Incremental	Bulk
NRS scale	0.4 (0.8) A	0.2 (0.4) A	0.4 (0.9) A	0.3 (0.6) A
VAS scale (0-100)	5.9 (14.9) a	4.5 (11.6) a	8.0 (18.2) a	5.2 (13.1) a
VAS scale (0-10)	0.5 (1.5) [a]	0.4 (1.0) [a]	0.7 (1.7) [a]	0.5 (1.4) [a]

[a] *For each scale, the treatment groups were compared with Kruskall-Wallis and Mann-Whitney tests. Statistically similar groups are represented by the same uppercase letter (NRS scale), lowercase letter (VAS scale [0-100]), or superscript letter (VAS scale [0-100])* ($p=0.38$; $p=0.63$, and $p=0.67$, *respectively*).

Table 6: *Comparison of the Number of Patients (%) Who Experienced Spontaneous Postoperative Sensitivity During the One-Week Follow-Up According to the Characteristics of Dental Arches and Cavities*

Characteristic	Number of Sensitive Teeth (%)		*p*-Value ([a])
	No	Yes	
Tooth distribution			
Premolar	68 (87.2)	10 (12.8)	0.06
Molar	120 (75.9)	38 (24.1)	
Cavity depth			
3 mm	133 (81.1)	31 (18.9)	0.51
4 mm	55 (76.4)	17 (23.6)	
Number of restored surfaces			
1 + 2 faces	172 (82.3)	37 (17.7)	0.01
3 + 4 faces	16 (59.3)	11 (40.7)	

[a] *Chi-square test.*

studies that have compared the postoperative sensitivity in posterior cavities according to the size (depth and width), and they reported risks of postoperative sensitivity ranging from 25% to 40%, which is similar to the current results.[29,33,34,55]

According to the results of the present study, cavity depth did not show any impact on postoperative sensitivity. As the primary objective of this study was to evaluate the postoperative sensitivity of different placement techniques in posterior composite resin, only patients with a minimum cavity depth of 3 mm were included in the present study. Therefore, most of the cavities restored in the current study had a cavity depth varying from 3 to 5 mm, and this was probably responsible for the similarity of results.

However, a closer view of the studies that evaluated the relationship between cavity size and postoperative sensitivity provides controversial results.[29,34,56-58] This is likely related to the arbitrary classification of the cavity depth, which is usually based on the operator experience,[29] as there is lack of strict guidelines for defining the cutoff points for deep and shallow cavities.[59]

On the other hand, the number of surfaces restored had a significant impact on postoperative sensitivity, and this finding is in agreement with previous studies.[55-58,60,61] The increase in the removal of dental structure[33] and the difficulties faced by clinicians when restoring large preparations[60] may be the reason for an increased rate of postoperative sensitivity when the cavity involves more than two surfaces.

After one week, the risk of postoperative sensitivity was very low, as previously demonstrated by a meta-analysis of clinical studies,[30] indicating that postoperative sensitivity generated immediately after placement of a restoration appears to be transient, as previously demonstrated by histological studies in deep cavities.[62,63] The immediate postoperative sensitivity might be the result of trauma produced by bur cutting of the dentin substrates as well as those related to material polymerization.

In the present study, only spontaneous postoperative sensitivity was measured. This is in accordance with a recent systematic review and meta-analysis that compared the type of adhesive strategy on the risk and intensity of postoperative sensitivity in posterior composite resin restorations.[31] In that review, approximately 50% of the studies included in the meta-analysis assessed postoperative sensitivity by asking patients whether they experienced spontaneous postoperative sensitivity during a specific time frame.[31] Although the use of a stimulus to assess the risk and intensity of postoperative sensitivity has been used in some studies,[64-67] these approaches are especially important when evaluating pulp vitality rather than postoperative sensitivity.

There is a widespread belief among clinicians that self-etch systems lower the risk of postoperative sensitivity, as they do not remove but rather incorporate the smear layer in the hybridized complex with the advantage of being less technique sensitive.[68] Although there is a biological plausibility behind this belief with some clinical studies reaching this conclusion,[28,29] the perception that self-etch adhesives cause less postoperative sensitivity than etch-and-rinse systems seems to be more anecdotal than an evidence-based finding,[69] as other clinical trials do not support this trend.[64,67]

Indeed, the results of the present investigation are in line with a recent systematic review of the literature,[31] and therefore we could not reject the second null hypothesis. That systematic review concluded that the type of adhesive strategy used in bonding procedures in posterior composite resin restorations does not influence the risk and intensity of postoperative sensitivity immediately after the restorative procedure.[31]

Finally, one should report the limitations of the present study design. The present study was conducted in a university setting in which restorations are placed under ideal conditions to produce restorations as near perfect as possible. Calibrated

and experienced operators with a deep knowledge of the techniques and materials placed the restorations without time constraints. Additionally, moisture control was usually done with rubber dam isolation, preventing contamination of the operative field. Private clinicians must provide care with an eye toward minimizing the length of the appointment. A shorter appointment is more comfortable for the patients, it constrains costs, and it maintains a reasonable profit level. Additionally, just one type of bulk-fill material was evaluated, which prevents us from generalizing the findings of the present study to other bulk-fill materials available on the dental market.

The university setting is more appropriate for determining a material's optimal performance, while a practice-based setting investigates a material's typical performance. Therefore, further clinical studies using a practice-based design should be conducted to highlight whether the current results are applicable to less-than-ideal conditions.

CONCLUSION

The overall risk of immediate postoperative sensitivity was 20.3% (95% CI 15.7-25.9), and it was not affected by the adhesive strategy (etch-and-rinse/self-etch) or the filling technique (incremental/bulk).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the State University of Ponta Grossa. The approval code for this study is 109.846.

Conflict of Interest

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

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Laboratory Research

Margin Integrity and Secondary Caries of Lined or Non-lined Composite and Glass Hybrid Restorations After Selective Excavation *In Vitro*

F Schwendicke • JLM Kniess • S Paris • U Blunck

Clinical Relevance

Using glass hybrid or lining a cavity before restoring it with composite resins could decrease margin integrity. This might not be relevant for risk of secondary caries.

SUMMARY

Objectives: For deep carious lesions, selective carious tissue removal (leaving soft dentin close to the pulp) is suggested. Afterward, different restoration materials, such as resin composites or glass hybrids (GHs), can be placed. Many dentists also apply setting or non-setting calcium hydroxide liners before restoration. We compared margin integrity and susceptibility for secondary caries in differently restored premolars *in vitro*.

Methods: In 48 extracted human premolars, artificial residual lesions were induced on pulpo-axial walls of standardized cavities. Teeth were restored using a GH (Equia Forte) or adhesively placed resin composite restoration (OptiBond FL and Tetric EvoCeram) without any liner (RC), resin composite restoration with a non-setting calcium hydroxide liner (RC_NCH), or resin composite restoration with a setting calcium hydroxide liner (RC_SCH). After thermomechanical cycling, groups (n=12) were compared regarding their gingivocervical margin integrity (proportion of irregularities, microgaps, gaps >5 μm, overhangs). Teeth were then submitted to a continuous culture *Lactobacillus rhamnosus* biofilm model. After 14 days, bacterial numbers in biofilms, along tooth-restoration margins and mineral loss (ΔZ) of secondary lesions, were determined.

Results: GH and RC_NCH showed significantly higher proportions of irregularities than RC

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and RC_SCH ($p<0.05$/Mann-Whitney). GH also showed significantly more gaps than alternative restorations ($p<0.05$). Bacterial numbers and ΔZ did not differ significantly between groups ($p>0.05$).

Conclusions: GH and composites lined with non-setting calcium hydroxide showed reduced margin integrity compared with non-lined composites or composites lined with setting calcium hydroxide. This did not increase susceptibility for secondary caries.

INTRODUCTION

For deep carious lesions in teeth with vital pulps, selective (incomplete) carious tissue removal is recommended, leaving soft dentin in proximity to the pulp to avoid pulp exposure and postoperative pulp complications. Then, an adhesive restoration is placed, which seals, and thus inactivates, residual bacteria. The resulting residual carious dentin is soft, is less elastic, and allows only very limited bonding of dental adhesives in the lesion area,[1] all of which are feared to compromise the integrity and stability of the placed restoration. This, in turn, could limit the longevity of the restoration, leading to fracture or reduced margin integrity, which would presumably increase the susceptibility for secondary caries.[2,3]

Consequently, a variety of strategies have been developed to bond to this carious dentin and to restore the resulting extended cavity.[1,4,5] Besides these restorative challenges, dentists also aim to reduce pulpal risks, for example via placing a cavity liner, which supposedly protects the pulp and reduces postoperative pulp complications. Moreover, such liners should also exert antibacterial or remineralization effects.

The most commonly placed lining material is calcium hydroxide (CH), which has been shown to provide remineralization *in vitro*[6] and clinically.[7,8] CH is also antibacterial and induces reactionary dentin development.[9-11] Two different application forms of CH are available, setting and non-setting CH. Setting CH sets after mixing with salicylate esters via a chelating reaction, resulting in a moderately stable lining layer with low compression strength; it releases fewer calcium and hydroxyl ions than non-setting CH,[12,13] which potentially exerts higher antibacterial effects. Both materials are susceptible for degradation via acid-etching[14] and dissolve over time, leaving voids and tunnels prone to bacterial recolonization.[15,16] They are conventionally covered with a self-conditioning material (like resin-modified glass ionomers) before placing an adhesive restoration (eg, acid-etching or applying self-etch adhesives). In summary, CH are widely used, though their application might mechanically compromise the subsequent restoration (eg, its margin integrity or fracture resistance), the clinical impact of which remains unclear at present.

To avoid such lining material and the required additional treatment steps, but nevertheless to remineralize the residual lesion, a variety of restoration materials have been proposed, for example fluoride-doped and fluoride-releasing composites, or glass ionomer cements (GICs). The latter have been shown to remineralize dentin *in vitro* and *in vivo*.[17-23] However, GICs have mainly been placed temporarily over residual lesions, as their limited flexural strength does not allow for their use to permanently restore extended cavities. Recent advances in this material class, however, have widened the indication spectrum of GICs, and one very recent development was the introduction of glass hybrids (GHs). These are reinforced GICs, with a second, smaller, and more reactive silicate particle and higher-molecular-weight acrylic acid molecules, which supposedly increase matrix cross-linking. This, in turn, is thought to improve the material's flexural strength. Covering these restorations with a resin layer is supposed to further improve wear resistance and esthetic appearance. It remains unclear if GH may truly be used to restore extended cavities, as the margin integrity and fracture resistance of teeth restored with GH are unknown. Moreover, the secondary caries susceptibility of teeth restored with GH is not clear, which could be linked to restoration margin characteristics of these materials and their fluoride release.

Therefore, the present study aimed to assess the margin integrity and secondary caries susceptibility of lined and non-lined composite resin and GH restorations. We hypothesized that lining materials significantly decrease margin integrity of composite restorations after selective excavation, and we also assumed that GH would have significantly lower margin integrity than composite. In line with this, we hypothesized that mineral loss of biofilm-induced secondary caries lesions is significantly larger adjacent to lined compared with non-lined composites and with GH compared with composite.

METHODS AND MATERIALS

Experimental Setup

We compared four groups of restoration strategies: (1) Resin composite without liner, (2) resin composite

(RC) on top of a setting CH liner placed at pulpal cavity walls, (3) RC placed on top of a non-setting CH, and (4) GH. We assessed three parameters. First, margin integrity after thermomechanical cycling was determined using replica analysis via scanning-electron microscopy. Second, restored teeth were then submitted to a continuous-culture single-species biofilm model, and bacterial numbers in biofilms developed on margin areas were enumerated. Last, secondary lesions were assessed using microradiography.

Specimen Preparation

A sample size of 12 teeth per group had been determined a priori based on a previous study using a similar design.[24] Forty-eight extracted human upper second premolars obtained with informed consent under an ethics-approved protocol (EA4 102/14) were selected according to their mesial-distal (mean [range] = 6.5 [6.3/6.7] mm) and buccal-lingual width (8.7 [8.5/8.9] mm), with a maximum deviation of 0.2 mm from the means in each dimension set as the limit. Teeth were cleaned, examined for cracks, and embedded (Technovit 4071, Heraeus Kulzer, Hanau, Germany) in chromed brass tubes (15 mm length, ø15 mm; Bauhaus, Belp, Switzerland) 2 mm below the cementoenamel junction using a gauge. Standardized cavities were prepared using water-cooled copy-milling (Celay, Mikrona, Spreitenbach, Switzerland). Minor adjustments were performed with rotating instruments. Cavity surfaces were controlled for cracks or abnormalities using a stereomicroscope (Stemi Zoom, Zeiss, Oberkochen, Germany). Teeth were then covered with nail varnish (43K, Manhattan, Mainz, Germany), with two rectangular windows (1.5 × 2 mm) left unprotected on the mesial and distal pulpal-axial walls (Figure 1).

Teeth were submitted to an established protocol to induce artificial residual caries lesions resembling those remaining after selective excavation using the criterion of leathery dentin remaining in proximity to the pulp.[25] Briefly, teeth were exposed to an acetic acid solution containing 50 mM acetic acid, 3 mM $CaCl_2 \times 2\ H_2O$, 3 mM KH_2PO_4, and 6 μM methylhydroxy-diphosphonate (pH 5.30, 37°C) for 12 weeks. Depths and mineral loss of induced lesions were determined on 10 random samples via microradiography (see the sections that follow) after the conclusion of the experiment. Artificial residual lesions had a mean (standard deviation) depth of 76 (27) μm and a mean ΔZ of 1575 (736) vol%×μm.

After demineralization, the nail varnish was mechanically removed, and surfaces were checked again. A Tofflemire matrix was placed, and cavities were restored according to one of four protocols:

1. Tetric EvoCeram RC (Ivoclar Vivadent, Schaan, Liechtenstein), placed incrementally in laminate technique, after conditioning the cavity using phosphoric acid 37% (Orbis, Münster, Germany) and chlorhexidine 2% (Charité Hausapotheke, Berlin, Germany) for rewetting, and placement of OptiBond FL (Kerr Italia, Salerno, Italia). Adhesive treatment was performed as follows: primer application for 30 seconds, evaporation of the solvent, and application of bond. After each increment, light-curing was performed for 20 seconds using a light-emitting diode curing light (Valo, Ultradent, Salt Lake City, UT, USA) with an intensity of 1400 mW/cm^2.
2. Tetric Evo Ceram, as described, with non-setting CH (UltraCal XS, Ultradent, Cologne, Germany) placed prior to cavity conditioning as a liner (RC_NCH). CH was only placed on the artificial lesion and was covered with a thin layer of Vitrebond Plus (3M ESPE, St Paul, MN, USA) which was light-cured for 20 seconds.
3. Tetric EvoCeram, as described, with setting CH (Kerr Life, Scafati Salerno, Italy) being used as liner (RC-SCH), which was also covered with Vitrebond Plus before restoring the cavity.
4. GH (Equia Forte, GC, Tokyo, Japan), placed in a bulk, after conditioning with polyacrylic acid (Equia Cavity Conditioner, GC), according to manufacturer instructions. Afterward, GH was covered with resin coating (Equia Coat, GC) on the occlusal surface, followed by light-curing for 20 seconds. Note that gingivocervical margins were not covered, as this would not be clinically possible in most circumstances.

The restorations were polished (SoFlex and Greenie/Brownie, Henry Schein, Melville, NY, USA) after placement and checked under loupes and a stereomicroscope (Stemi Zoom).

Thermomechanical Cycling

Teeth were submitted to thermal cycling of 10,000 cycles between 5°C-55°C, with 12 s/30 s dwell/equilibration time, respectively, in distilled water (liquid cycler, Haake, Karlsruhe, Germany). Afterwards, 1.2 × 10^6 mechanical cycles were performed,[26] with a ceramic ball (ø = 5 mm; Steatite, Hoechst, Wunsiedel, Germany) being loaded onto the occlusal surface 1.5 mm with a load of 5 kg[27] in a

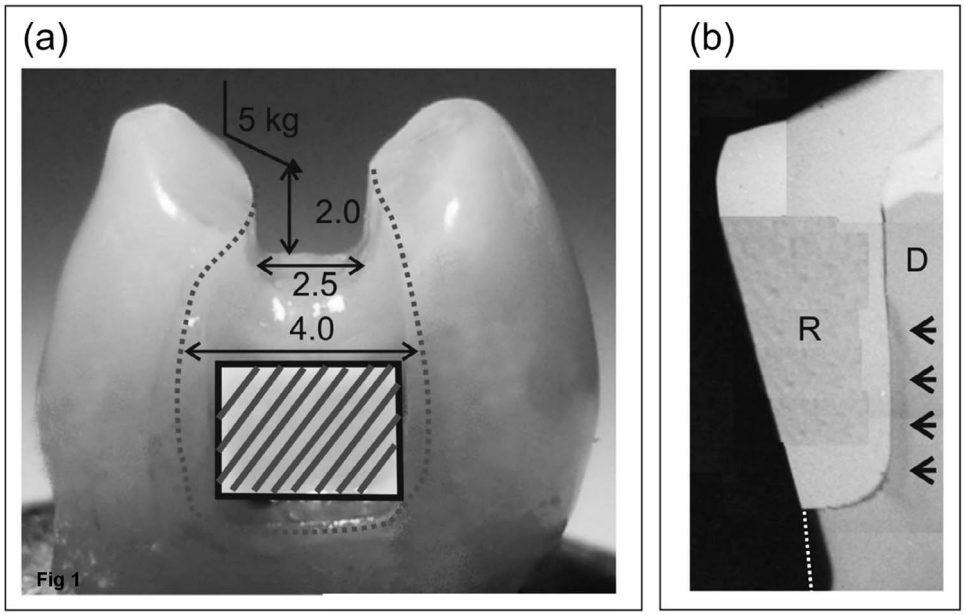

Figure 1. *Specimens. (a): Standardized cavities were prepared using copy-milling. Since teeth were chosen according to their buccal-lingual and mesial-distal dimensions, the remaining cuspal-supporting hard tissue was standardized as well. Cervical margins were located 1.0 mm above (mesial) or below (distal) the cementoenamel junction. Artificial rectangular lesions (1.5 × 2 mm) were created at pulpo-axial walls (hatched grey area). After placement of a composite restoration (dotted line), teeth were submitted to mechanical cycling using a ceramic ball loaded 1.5 mm below the cuspal aspect on the inner incline of the palatal cusp with a force of 5 kg (black arrow). (b): Microradiograph of a restored sample. The restoration (R), dentin (D), and the artificial residual lesion (arrow heads) are shown. On the outer dentin surface, a secondary caries "outer" lesion induced during biofilm challenge is detected (the dotted line indicates the original outline of dentin). Note that the microradiograph is composed of several single images, leading to (quadrangular) artifacts (as can be noted mainly in the restoration area).*

dual-axis chewing simulator (Kausimulator CS-4.8, Willytech, Feldkirchen-Westerham, Germany) in distilled water.

Quantitative Margin Analysis

Gingivocervical margins were assessed using scanning electron microscopy as described.[28] Briefly, margins were replicated using polyvinyl-siloxane impression material (Honigum, DMG, Hamburg, Germany) and epoxy resin (Stycast 1266, Henkel, Westerlo-Oevel, Belgium), and assessed both directly after finalization of the restoration and after thermomechanical cycling. Margin adaptation was evaluated at 200× magnification (AMRAY 1810, Amray, Bedford, MA, USA) using defined criteria[29]: Perfect margin, irregularities or submarginations, microgaps (hairline cracks), distinct gaps >5 μm, and overhangs/positive ledges.

Secondary Caries Model

To assess the susceptibility for caries adjacent to the restoration, teeth were covered using nail varnish, leaving a window (2×2 mm) on the mesial and distal gingivocervical margins uncovered. A computer-controlled continuous-culture biofilm model[30] was used for bacterial demineralization. Overnight cultures of *Lactobacillus rhamnosus* (DSM 20021) were prepared in deMan-Rogosa-Sharpe medium at 37°C. Teeth and bacterial suspension were daily incubated within the biofilm chamber at 100% humidity at 37°C for 30 minutes. After a 1-hour pause, the cycle started: specimens were provided with a flow of 100 mL sterile MRS supplemented with 2% sucrose and amphotericerin B for 45 minutes, followed by 200 mL sterile defined mucine medium[31] for 30 minutes using peristaltic single-canal pumps (Type PR1, Seko, Mainz, Germany) with a resting period of approximately 6 hours. Each cycle was performed five times a day. Once a day, 250 ppm sodium fluoride rinses were provided for 10 minutes. After 14 days, cultivation was concluded, and biofilms on the defined windows were removed, transferred to 0.9% sodium chloride, serially diluted from 10^{-1} to 10^{-7}, and plated onto deMan-Rogosa-Sharpe agar for enumeration of colony-forming units after 48 hours. Induced lesions adjacent to restorations were assessed using transversal microradiography.

Transversal Microradiography

Teeth were embedded in acrylic resin (Technovit) and divided midsagittally through the induced demineralized lesion. Two thin sections (200 μm) per tooth were prepared (band saw 300cl; Mikroschleifsystem 400 CS, abrasive Paper 800 and 1200). A nickel-filtered copper X-ray source (PW3830,

Pananalytical, Kassel, Germany) operating at 20 kV and 20 mA was used to obtain radiographs. Films (35 mm B/W positive, Fujifilm, Tokyo, Japan) were exposed for 10 seconds and developed under standardized conditions according to the manufacturer's recommendations. Microradiographs were analyzed using a digital-image-analyzing system (CFW 1312M, Scion, Frederick, MD, USA) interfaced with a universal microscope (Axioplan 60318, Zeiss, Oberkochen, Germany) and a personal computer (Transversal Microradiography for Windows 5.25, UMCG, Groningen, The Netherlands), with integrated mineral loss (ΔZ) being assessed. We controlled for the presence of both outer and wall lesions,[32] but no wall lesions could be found. In case the lesion was heterogeneous, for example, deeper in proximity to the restoration, the deepest section of the lesion was analyzed. Of the two assessed lesions per margin, means were calculated for further analysis.

Statistical Analysis

Data were analyzed using SPSS 20 (IBM, Armonk, NY, USA). Normal distribution was checked using the Shapiro-Wilk test. Group-wise comparisons were performed using the Mann-Whitney U test. Level of significance was set at $\alpha=0.05$.

RESULTS

Regarding margin integrity (Figure 2), GH and RC_NCH showed significantly higher proportions of irregularities along enamel margins than RC and RC_SCH ($p<0.05$; Mann-Whitney U test). GH also showed significantly more gaps along dentin and enamel margins than alternatives ($p<0.05$).

Bacterial numbers (Table 1) did not differ significantly between groups ($p>0.05$).

Mineral loss of secondary caries lesions was significantly higher in dentin than enamel ($p<0.05$) but did not differ significantly between groups (Figure 3). No wall lesions were detected in any of the groups, that is, all lesions were outer lesions.

DISCUSSION

When treating deep lesions, dentists are challenged to manage pulpal vitality (which is why many place cavity liners for pulp protection) and to restore the resulting extended cavity.[11] The latter is especially relevant when performing selective carious tissue removal, leaving soft residual carious dentin in proximity to the pulp, which might compromise the margin integrity of restorations and therefore affect treatment success or tooth/restoration survival. The present study investigated whether cavity liners affect the integrity of composite restorations and assessed the suitability of a new material class, GHs, for restoring cavities after selective excavation. We found GHs to have relatively poor gingivocervical margin integrity, with a large proportion of irregularities or distinct gaps. Moreover, we found non-setting CH lining to detrimentally affect margin integrity. We thus accept our first hypothesis. However, and clinically probably more relevant, materials did not differ significantly in their susceptibility for secondary caries or biofilm accumulation in cervical areas. We therefore refute our second hypothesis.

The observed margin integrity behavior of GHs is closely related to the composition of the material, which is largely unpolishable. In conventional GICs, this leads to unacceptable wear, which is why these materials are not recommended for permanent restorations in most countries. For GHs, the load-bearing surface is covered with a methacrylate resin, which has been found to significantly decrease wear.[33] However, this resin is not applied on proximal surfaces, which is why certain margin irregularities are most likely unavoidable in gingivocervical proximal margins. However, we also found significant proportions of distinct gaps after thermocycling, which might indicate local debonding. In contrast, non-lined RC showed a perfect margin integrity, with only few teeth showing any imperfections along the margin in both enamel and dentin. This once more confirms that excellent bond and restoration integrity can be achieved after selective excavation with current adhesive systems. Placing a non-setting CH liner seems to negatively affect margin integrity, with a large proportion of enamel and dentin margins showing irregularities. It seems that the permanently soft lining material decreases the mechanical support of the composite restoration, leading to a possibly different bending behavior under load and eventually decreased margin integrity. It should be noted that this was found even while liners were placed only on the lesion area, that is, very locally, which might not always be clinically possible. This possible detrimental effect of CH is especially remarkable, as the positive attributes of CH lining, such as remineralization or antibacterial effects, are also increasingly questioned.[8,34-37] Thus, CH lining could have only very limited benefit for the pulp but negative effects on the restoration,[38-40] while requiring an additional

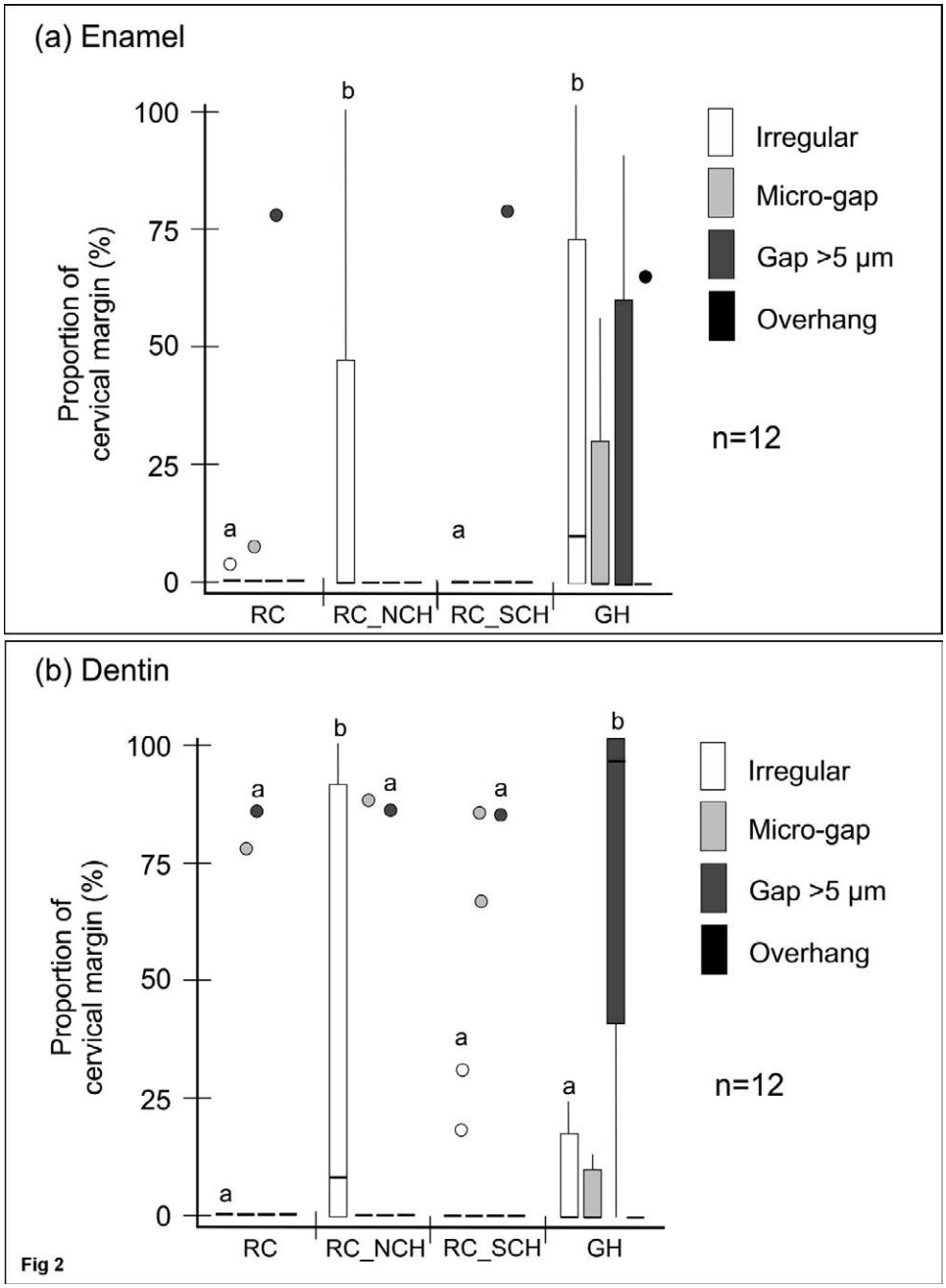

Figure 2. *Margin integrity of different restorations after thermomechanical cycling. Gingivo-cervical margins in (a): enamel and (b): dentin were assessed for their integrity, with the proportion of irregular margins (white), margins with microgaps (light grey), or margins with distinct gaps (dark grey) as well as overhangs/ positive ledges (black) being quantified. Note that the percentage of perfect margins is not given, as it results from these four margin categories. Different superscript letters indicate significant differences between groups (*$p<0.05$*, Mann-Whitney U test). Letters are only provided for categories with significant differences between groups. n=number of specimens per group. Box and line: Interquartile range and median; whiskers: range; circles: outliers. GH, glass hybrid; RC, resin composite restoration without any liner; RC_NCH , resin composite restoration with a non-setting calcium hydroxide liner; RC_SCH, resin composite restoration with a setting calcium hydroxide liner.*

treatment step, that is, increased efforts. GH, in contrast, is easy to apply even under moderately adverse conditions, and was found to provide significant remineralization via fluoride and strontium release.[41]

Such release could also exert antibacterial effects and reduce bacterial colonization along margins and prevent or slow down the development of secondary lesions. We did not find such secondary caries preventive effect of GH. In our study, margin behavior generally did not correlate with mineral loss of lesions adjacent to restoration. It seems that while microscopic margin integrity assessment is a sensitive technique to compare materials, it has limited external validity. As shown in a previous study, the observed irregularities or interfacial gaps do not translate into the development of true "secondary" lesions (constituting an outer and a wall lesion). Instead, all induced lesions were outer lesions adjacent to a restoration, possibly without any causal association with the restorative material or integrity.[32,42] This, however, might be specific to

Table 1: *Bacterial Numbers in Biofilms at Restoration Margins*[a]

Group	Enamel	Dentin
RC	8 (3/43)	26 (8/79)
RC_NCH	7 (4/26)	13 (9/19)
RC_SCH	11 (5/21)	11 (9/46)
GH	18 (13/49)	28 (20/43)

Abbreviations: GH, glass hybrid; RC, resin composite restoration without any liner; RC_NCH , resin composite restoration with a non-setting calcium hydroxide liner; RC_SCH, resin composite restoration with a setting calcium hydroxide liner.
[a] *Biofilms were assessed at gingivocervical enamel and dentin margins in 12 samples per group. Median and 25th/75th percentiles of $10^5 \times$ colony-forming units are given. Colony-forming units did not differ significantly between groups ($p>0.05$, Mann-Whitney U test).*

the present study or *in vitro* investigations, where margin integrities are overall relatively high, that is, even distinct gaps measure only few micrometers. It might be that larger gaps are required for the formation of a proper "wall lesion"[2,3,43] via leakage of bacteria or acids along the restorative interface.[32,44] Recent studies also highlighted that masticatory loading might influence secondary caries lesion induction, possibly via a "pumping effect." No such loading was provided in our study during the biofilm phase. Thus, in a clinical setting, true secondary lesions could be more likely.[45] In such a setting, long-term margin integrity and secondary lesion induction will differ not only between materials but also between patients due to individually different load strengths and caries risks/[45-49]

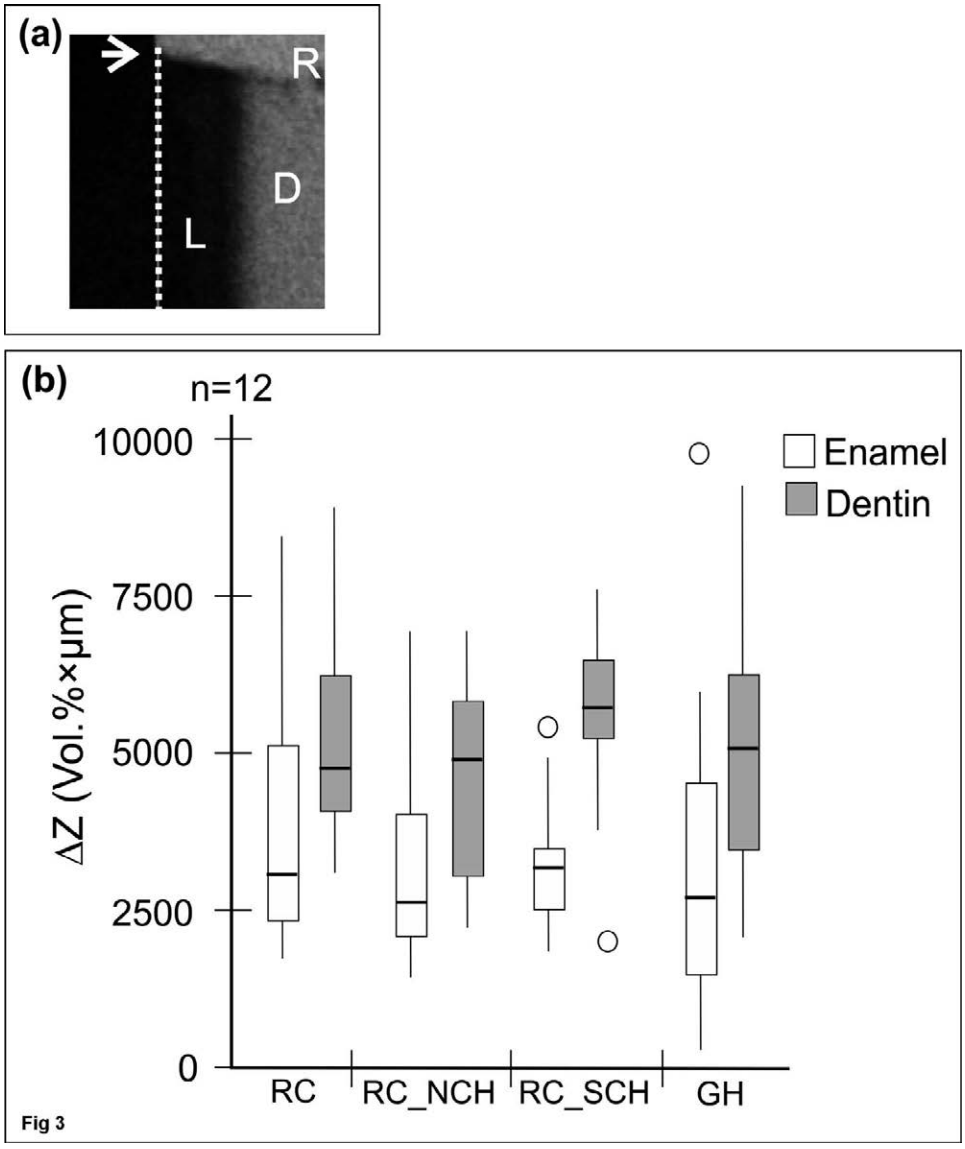

Figure 3. *Mineral loss adjacent to restorations. (a): Microradiographs were analyzed for mineral loss adjacent to restorations (R). We analyzed lesions in enamel and dentin (D), the latter being shown here. The dotted line indicates the lesion surface. In no sample was a wall lesion; all lesions were outer lesions (L). (b): Mineral loss (ΔZ) of enamel (white) and dentin (grey) carious lesions adjacent to restorations. No significant differences between groups were identified ($p>0.05$; Mann-Whitney U test). n=number of specimens per group. Box and line: Interquartile range and median; whiskers: range; circles: outliers. GH, glass hybrid; RC, resin composite restoration without any liner; RC_NCH, resin composite restoration with a non-setting calcium hydroxide liner; RC_SCH, resin composite restoration with a setting calcium hydroxide liner.*

The present study has a number of limitations. First, the cavities were standardized via copy-milling, which reduces variability between samples but results in cavities that might be less undermining and extended than real-life deep cavities. Moreover, liner application was not fully standardized, which could have contributed to the observed variance. Second, while the protocol used to induce residual lesions has been found to generate lesions with similar mechanical and mineral loss characteristics as natural residual lesions,[25] the experimental setup does not account for any remineralization from the pulp (we did not investigate mineral gains induced by restorative or lining materials in this study). Such remineralization could alter mechanical properties with time (which would not change bond strengths but the support of the restoration against mechanical loading). Thus, the external validity of the chosen setup is limited. Similarly, we simulated premolars, where most lesions occur proximally first. In this case, pulp exposure is most likely at pulpoaxial walls, which is where residual carious dentin was simulated. If lesion location is different, for example, occlusal, or the excavation is terminated earlier than simulated by our protocol (which would result in more soft dentin being left beneath the restoration), one might expect different material behaviors. Our findings thus cannot be transferred to such situations. Third, the used biofilm model was comprised of *L. rhamnosus* mono-species biofilms only, while multispecies biofilms or *in situ* analysis might yield more realistic findings. Additionally, mineral loss was not assessed along the whole margin, only in two sections of each tooth, which do not necessarily represent the area of greatest marginal imperfection. Fourth, our findings of statistical nondifference between materials with regards to secondary caries might be attributed to a lack of power. However, considering that for reaching significant differences in mineral loss in enamel, 4500 samples would be needed according to power calculations, while 87 specimens per group would be required to demonstrate significant differences in dentin, the clinical relevance of these differences might be limited. Last, we only assessed one restorative parameter, margin integrity and the resulting susceptibility for biofilm formation and secondary caries. Future studies should compare differently restored teeth for static fracture or fatigue resistance and complement these analyses with finite element modeling to allow a better understanding of the effects of lining among other issues.

CONCLUSIONS

In conclusion, GH or composite restorations that were lined with non-setting CH showed inferior margin integrity compared with non-lined composites in cavities resulting from selective excavation. No such disadvantage was shown for setting CH. Differences in margin integrity did not translate into different susceptibility for secondary caries. Clinical decision making should weigh the efforts for placing liners and different restoration materials against the resulting pulpal and restorative outcomes.

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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 Charite Berlin. The approval code for this study is EA4 102/14.

Conflict of Interest

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

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The Use of Resin Composite Layering Technique to Mask Discolored Background: A CIELAB/CIEDE2000 Analysis

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Clinical Relevance

Esthetic resin composite restorations can be achieved over a discolored tooth if the layering technique using different combination shades is correctly applied.

SUMMARY

The purpose of this study was to evaluate the ability of three resin composite systems to mask a severely discolored background by the application of a layering technique through CIELAB and CIEDE2000 analysis. Ninety 1.5-mm-thick disc specimens were produced from three different resin composite restoration systems: IPS Empress Direct (Ivoclar Vivadent), Charisma Diamond (Heraeus Kulzer), and Filtek Z350 XT (3M-ESPE). The specimens were divided into groups according to the restoration system and the resin composite shade combination used for the layering technique (enamel, body, and dentin shades). Color measurements were performed by a reflectance spectrophotometer (SP60, EX-Rite) against a C4 shade background and an inherent color background, which simulates a severely discolored background and a tooth surface with no discoloration, respectively. The total color difference between both color measurements was calculated by CIELAB (ΔE^*_{ab}) and CIEDE2000 (ΔE_{00}) formulas. The mean ΔE_{ab}^* and ΔE_{00} values were analyzed by analysis of variance (general linear models) and Tukey's post hoc tests (α=0.05). Three groups presented clinically acceptable color difference values ($\Delta E^* \leqq 3.46$ and $\Delta E_{00} \leqq 2.25$): 1.5 mm dentin, 1.0 mm dentin/0.5 mm body, and 1.0 mm dentin/0.5 mm enamel; ie, all the groups from the Z350 XT restoration system. The resin composite layering technique is an effective way to mask severely discolored backgrounds. The Filtek Z350 XT system was the only restoration system capable of masking the C4 background.

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INTRODUCTION

Tooth discoloration is one of the main reasons for esthetic dissatisfaction regarding patient smile appearance. The etiology of dental discoloration is not completely understood and may affect an individual tooth or a group of teeth. Changes in individual tooth color are commonly caused by blood extravasation, trauma, or the outcome of endodontic procedures.[1] Furthermore, contaminants, such as metallic ions from amalgam restorations, and root resorption are also associated with tooth discoloration.[2]

Single tooth discoloration is considered a complex and challenging clinical situation, which requires a correct diagnosis, an understanding of the etiology, and an adequate treatment planning for a successful esthetic outcome. Despite the variety of bleaching approaches and possibilities, a satisfactory resolution is not always guaranteed in some clinical situations, especially when the etiologic causes are dentin impregnated by amalgam ions or pulp bleeding.[3] Direct and indirect resin composite restorations are a simple and fast solution to cover up darkened dental substrates that did not respond to bleaching. Resin composite restorations are a more cost-effective, viable, and comfortable treatment option for the patients.[4] Advantages, such as reversibility and a conservative procedure based on the adhesive technique, increase the acceptance of the treatment by the patients and dentists.[5,6]

Manufacturers are constantly improving the color properties of their products. Despite the improvements, composites are translucent materials, and the esthetic results can be impaired, depending on the intensity of the dental discoloration. Translucency is defined as the intermediate state between complete opacity and complete transparency.[7] The underlying discolored structure may affect the final value and lightness of the composite restoration. This situation leads to the perception of a chromatic mismatch between the restoration and the adjacent tooth structures.[4,8]

When the layer-to-layer technique is applied, an opaque and/or a body-shade can be used to cover up more intense tooth color defects, whereas a more translucent shade can be applied as a last covering layer. The correct application of the layering technique should minimize color discrepancies, such as loss of lightness, and also may promote compensatory changes in the final restoration, which may mask the discolored underlying tooth substrate.[4,9-11]

Optical properties and masking abilities of opaque-shade resin composites are well documented in the literature.[4,7,12] However, more information concerning the masking ability of different resin composite shade combinations are necessary; the effectiveness of the multilayering technique on a discolored background is still unclear. One question still needs to be answered: is it possible to mask severely discolored backgrounds by the multilayering technique using different resin composites shade combinations? The purpose of this study was to evaluate the ability of three resin composite systems to mask a severely discolored background using different shade combinations by the layering technique. The null hypothesis was that there were no differences in masking ability among the tested resin composite restoration systems and shade combinations.

METHODS AND MATERIALS

Specimen Preparation

Three resin composite restoration systems (IPS Empress Direct, Ivoclar Vivadent, Schaan, Liechtenstein; Charisma Diamond, Heraeus Kulzer, Hanau-Hessen, Germany; Filtek Z350 XT, 3M-ESPE, St. Paul, MN, USA) were tested in this study. The systems are available commercially with enamel shades (more translucent) and dentin shades (less translucent) as basic options. Both Filtek Z350 XT and IPS Empress Direct follow the Vitapan Classical shade guide. The Charisma diamond shade guide is an adaptation from Vitapan Classical, being that the opaque-shades are compatible with more than one Vitapan shades. Additionally, the Z350 XT system offers a body shade with an intermediate opacity (between enamel and dentin translucency). The chemical composition, manufacturers, shade, and batch numbers of the materials used in this study are listed in Table 1.

Specimens were prepared by using a stainless steel split matrix with 0.5, 1.0, or 1.5 mm thickness and 11 mm inner diameter. Single-layered (SL) specimens were produced with a 1.5-mm-thick matrix. The composite was placed in one increment, and the top and bottom surfaces were flattened with Mylar strips and glass plates over 1 Kgf static load. Dual-layered (DL) specimens were built from a previously obtained 1.0 mm increment, placed inside a 1.5-mm-thick matrix. The resulting 0.5 mm depth cavity was then filled with other shades of the composites and light-cured. Triple-layered (TL) specimens were prepared following similar procedures, using 0.5-, 1.0-, and 1.5-mm-thick matrixes. The device used for specimen preparation is presented in Figure 1A-C. Each layer was light-cured

Table 1: *Composition and Information of Studied Restoration Systems*

Restoration system	Composition[a]	Manufacturer	Shades	Batch number
IPS Empress Direct	Dimethacrylate, Ba-Al-SiO4 glass silicate, oxide silicates, YbF3	Ivoclar Vivadent, Schaan, Liechtenstein	A1 Enamel A1 Dentin	A1-010040 OL-010030
Z350 XT	Bis-GMA, UDMA, TEGDMA, Bis-EMA, PEGDMA, BHT, silcate, zirconia	3M-Espe, St. Paul, MN, USA	A1E A1B A1D	1415300268
Charisma Diamond	UDMA, TCD-DI-HEA, Ba-Al-F glass silicate, YbF3, SiO2	Heraeus Kulzer GmbH, Hanau-Hessen, Germany	A1 Universal Opaque Light	A1-010040 OL-010030

[a] *Data provided by manufacturers. Abbreviations: Bis-GMA - bisphenol A-glycidyl methacrylate, BHT – butylated hydroxytoluene, YbF3 – ytterbium fluoride nanoparticles, SiO2 – Silicon dioxide, UDMA - urethane dimethacrylate, TEGDMA - triethyleneglycol dimethacrylate, Bis-EMA - bisphenol-A dimethacrylate, PEGDMA - polyethylene glycol dimethacrylate, TCD-DI-HEA - 2-propenoic acid, (octahydro-4,7 methano-1H-indene-5-diyl) bis(methyleneiminocarbonyloxy-2,1-ethanediyl) ester*

for 40 seconds with a 1200 mW/cm^2 irradiance-monitored light emitting diode (LED; Bluephase, Ivoclar Vivadent). Thereafter, the specimens were stored in distilled water for 24 hours at 37°C for additional composite translucency, lightness, and camphorquinone conversion, before performing the color measurements.

Ninety resin composite disc-shaped specimens (11 mm in diameter, 1.5 mm in thickness) were prepared and divided into 18 groups (n=5) to consider all possible layering shade combinations of each restorative system, including SL specimens, DL specimens, with a combination of two different opacities of the same system, and TL specimens, with combinations of the three opacities, which in this case, were available only in the Z350 XT system (Table 2).

Color Measurement

The color measurements were performed by a reflectance spectrophotometer (SP60, EX-Rite, Grand Rapids, MI, USA), against a C4 shade background (C4; L*=69.18; a*=6.80; b*=23.61) and an inherent color background (IC/Empress Direct: L*=82.99; a*=2.46; b*=17.88/Charisma: L*=81.87; a*=4.31; b*=18.55/Z350: L*=83.72; a*=1.52; b*=16.98). These two backgrounds simulate two different clinical conditions. The first one is a 2-mm C4-shade opaque ceramic tile and represents a severely discolored background. The latter is the inherent color of the resin composite and represents a tooth surface with no discoloration. Kamishima and others[13] reported that a 4-mm-thick resin-based composite disc is not affected by the underlying background color, regardless of shade and degree of translucency of the material, and could be considered the resin composite inherent color. Thus, the instrumentally measured color of a 4 mm resin composite disc could be considered the inherent color of the resin composite.[12] In the present study, a 4-mm-thick disk of each resin

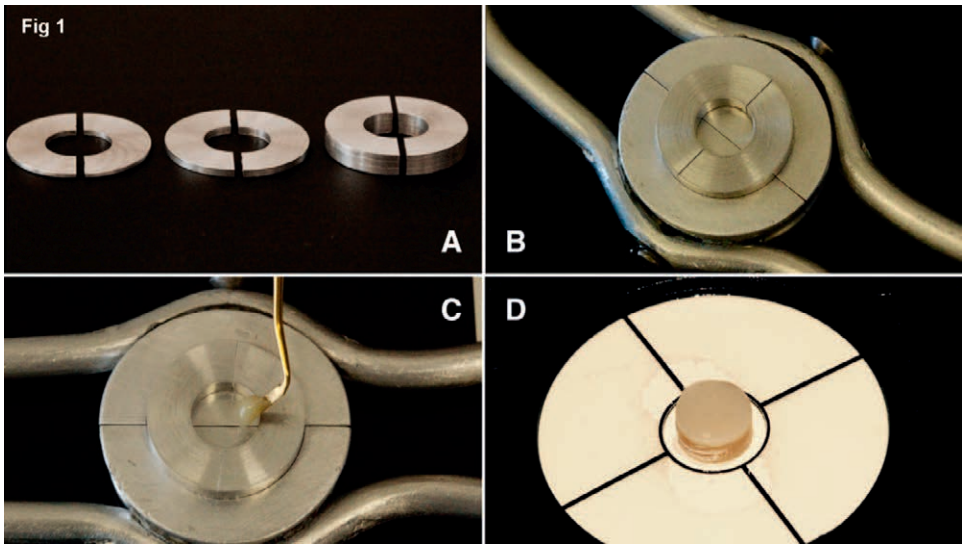

Figure 1. *Device used for specimen layering preparation and color measurements. (A): Split matrix with different thickness. (B): Split matrix positioned and stabilized inside the support base. (C): Resin composite being inserted inside the matrix. (D): Specimen positioned over the C4 background for color measurement.*

Table 2: *Possible Layering Combinations, Single Layered (SL), Dual Layered (DL), and Triple Layered (TL), of Resin Composite Systems*

Manufacturer	Shades	Layering technique (n=5)	
IPS Empress Direct (n=5)	A1E: A1 Enamel (E) A1D: A1 Dentin (D)	1.5 mm E	SL
		1.0 mm E + 0.5 mm D	DL
		0.5 mm E + 1.0 mm D	DL
		1.5 mm D	SL
Charisma Diamond (n=5)	A1U: A1 Universal E OL: Opaque light D	1.5 mm E	SL
		1.0 mm E + 0.5 mm D	DL
		0.5 mm E + 1.0 mm D	DL
		1.5 mm D	SL
Filtek Z350 XT (n=5)	A1E: A1 E A1B: A1 Body (B) A1D: A1 D	1.5 mm E	SL
		1.0 mm E + 0.5 mm B	DL
		1.0 mm E + 0.5 mm D	DL
		1.5 mm B	SL
		0.5 mm E + 1.0 mm B	DL
		1.0 mm B + 0.5 mm D	DL
		1.5 mm D	SL
		0.5 mm E + 1.0 mm D	DL
		0.5 mm B + 1.0 mm D	DL
		0.5 mm D + 0.5 mm B + 0.5 mm E	TL

composite system was made using the dentin shade of that system. The disc was used as the background for IC measurements. All the measurements were conducted according to the International Organization of Standardization (ISO) for color measurements.[14]

The CIELAB (ΔE^*_{ab}) color system[15] relative to standard illuminant D65 was used for the output of color measures. The CIE L*a*b* color system is a three-dimensional color measurement; L* refers to lightness, with values ranging from 0 (black) to 100 (white); a* and b* are considered chromatic coordinates: a* for red (+) and green (–) and b* for yellow (+) and blue (–). The specimens were positioned over the background with a coupling medium (glycerin) to simulate the oral environment color evaluation conditions[16] and then measured six times: three times against IC and C4 backgrounds (Figure 1D). All color measures followed the clinical layering pattern, with the enamel layers facing up, even when the enamel layer was the thickest layer in the shade combinations. Groups with body and dentin shade combinations followed the same evaluation as performed for enamel, with the body shade layer facing up for color reading. The average value of L*, a*, and b* of each background reading was calculated. The spectrophotometer was calibrated according to the manufacturer's instructions before conducting the color measurement.

The color difference of the same specimen against the backgrounds was calculated by the use of two different equations. The first one is the CIELAB color difference (ΔE^*_{ab}) equation, which was calculated as follows[15]:

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

where ΔL^*, Δa^*, and Δb^* refers to lightness, green-red, and blue-yellow differences of C4 and IC backgrounds color measurements.

The second is the CIEDE2000 color difference (ΔE_{00}), and it was calculated as follows[17]:

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

where $\Delta L'$, $\Delta C'$, and $\Delta H'$ are considered lightness, chroma, and hue differences between color measurements. K_L, K_C, and K_H are the parametric factors for viewing conditions and illuminating conditions influence, which in this study were set to 1.[17] R_T is the function for the hue and chroma differences interaction in the blue region. S_L, S_C, and S_H are the weighting functions for the color difference adjustment considering the location variation of L*, a*, and b* coordinates.[17,18]

Lower ΔE^*_{ab} and ΔE_{00} values indicate that the specimen is less sensitive to the influence of the

Table 3: *L*, a*, and b* Coordinate Mean Values Against IC and C4 Backgrounds and ΔE^*_{ab} and ΔE_{00} Color Difference Mean Values of All Studied Resin Composite Combinations*[a]

Brand	Shades combination	IC color measure			C4 color measure			ΔE* (SD)	ΔE_{00} (SD)
		L*	a*	b*	L*	a*	b*		
IPS Empress Direct	1.5 E	82.89	3.07	20.45	76.42	3.75	16.12	7.83^{a} (0.13)	5.26^{A} (0.12)
	1.0 E + 0.5 D	83.09	3.04	19.98	77.41	3.27	15.80	7.05^{b} (0.31)	4.62^{B} (0.24)
	0.5 E + 1.0 D	83.48	2.94	19.10	78.18	2.95	15.09	$6.64^{b,c}$ (0.10)	$4.32^{B,C}$ (0.04)
	1.5 D	84.78	2.78	19.53	80.31	2.54	15.38	$6.10^{d,e}$ (0.20)	$3.81^{D,E}$ (0.16)
Charisma Diamond	1.5 E	82.77	3.57	19.30	77.42	3.41	17,13	5.60^{f} (0.36)	$3.89^{D,E}$ (0.18)
	1.0 E + 0.5 D	82.76	3.57	18.86	77.43	3.30	16.83	$5.70^{e,f}$ (0.12)	$3.85^{D,E}$ (0.08)
	0.5 E + 1.0 D	83.56	3.80	18.61	77.89	3.57	16.44	$6.07^{d,e}$ (0.16)	$4.08^{C,D}$ (0.12)
	1.5 D	83.57	3.90	19.19	78.40	3.75	17.10	5.58^{f} (0.09)	3.73^{E} (0.07)
Filtek Z350 XT	1.5 E	83.60	1.46	16.48	77.66	2.96	13.10	7.00^{b} (0.25)	5.07^{A} (0.20)
	1.0 E + 0.5 B	82.79	1.60	16.29	77.72	2.78	12.73	$6.29^{c,d}$ (0.14)	4.50^{B} (0.11)
	1.0 E + 0.5 D	83.67	1.69	16.75	79.74	2.06	13.71	4.99^{g} (0.26)	3.31^{F} (0.20)
	1.5 B	82.83	1.91	18.09	78.53	2.60	14.36	$5.73^{e,f}$ (0.22)	$3.85^{D,E}$ (0.17)
	0.5 E + 1.0 B	82.67	1.91	17.07	78.09	2.75	13.51	$5.87^{d,e,f}$ (0.11)	$4.05^{C,D}$ (0.08)
	1.0 B + 0.5 D	82.77	2.02	18.14	79.62	2.03	15.31	4.23^{h} (0.12)	2.70^{G} (0.08)
	1.5 D	84.68	1.61	16.82	83.34	1.33	15.33	**2.03^{k} (0.15)**	**1.27^{J} (0.10)**
	0.5 E + 1.0 D	83.63	1.72	17.12	81.28	1.47	14.72	**3.30^{i} (0.20)**	**2.12^{H} (0.12)**
	0.5 B + 1.0 D	82.64	1.73	17.84	80.69	1.49	15.80	**2.83^{j} (0.26)**	**1.77^{I} (0.16)**
	0.5 E + 0.5 B + 0.5 D	82.17	1.68	16.43	79.49	1.63	13.76	3.78^{i} (0.05)	$2,44^{G}$ (0.04)

[a] *Bold ΔE^* and ΔE_{00} values are considered clinically acceptable. Means that do not share a letter are significantly different. Lowercase letters are related to comparisons among CIELAB (ΔE^*_{ab} mean values, and uppercase letters are related to comparisons among CIEDE2000 (ΔE_{00}) values).*

background color, and consequently, has a greater masking ability. The clinical acceptance threshold considered in this study for CIELAB calculation was $\Delta E^*_{ab} = 3.46$ and for CIEDE2000 calculation was $\Delta E_{00} = 2.25$. Any color difference value higher than these thresholds can be distinguished by an unskilled individual and cannot be considered as clinically acceptable.[14,18]

The mean ΔE^*_{ab} and ΔE_{00} values were analyzed by analysis of variance (general linear model) and Tukey's post hoc tests (α=0.05). Statistical analysis was performed using Minitab software (Minitab, Inc., State College, PA, USA).

RESULTS

The L*, a*, and b* coordinates values against each background and ΔE^*_{ab} and ΔE_{00} values of each shade combination are shown in Table 3. Three groups presented $\Delta E^*_{ab} \leq 3.46$ and $\Delta E_{00} \leq 2.25$. There was a statistically significant difference ($p<0.05$) between these three groups when they were compared with the other experimental groups: 1.5 mm dentin (ΔE^*_{ab}=2.03/ΔE_{00}=1.27), 1.0 mm dentin + 0.5 mm body (ΔE^*_{ab} =2.86/ΔE_{00}=1.77), and 1.0 mm dentin + 0.5 mm enamel (ΔE^*_{ab}=3.3/ΔE_{00}=2.12), all of them being part of the Z350 XT system. Empress Direct 1.5E (ΔE^*_{ab}=7.83) presented the highest ΔE^*_{ab} when analyzed through the CIELAB equation and when it was compared with the single-layered enamel Z350XT (ΔE^*_{ab}=7.00) and Charisma (ΔE^*_{ab}=5.60) groups ($p<0.05$). Comparisons among CIELAB values of experimental groups are shown in Figure 2.

Considering CIEDE2000 analysis, statistically significant differences were not found among Z350XT 1.5B (ΔE_{00}=3.85), Charisma 1.5D (ΔE_{00}=3.74), Charisma 1.5E (ΔE_{00}=3.89), and Empress Direct 1.5D (ΔE_{00}=3.81) ($p>0.05$). The combination of Z350XT 1B/0.5E (ΔE_{00}=4.05) also showed similar ΔE_{00} values compared with Charisma 1D/0.5E (ΔE_{00}=4.08) and Empress Direct 1D/0.5E (ΔE_{00}=4.32) ($p>0.05$). Overall ΔE_{00} value comparisons among all the study groups are presented in Figure 3.

DISCUSSION

Among all tested shade combinations and through both color difference analyses, three Z350 XT groups showed values under the clinical acceptance threshold, and these three groups were also statistically different compared with the other experimental groups ($p<0.05$). None of the shade combinations of Empress Direct and Charisma

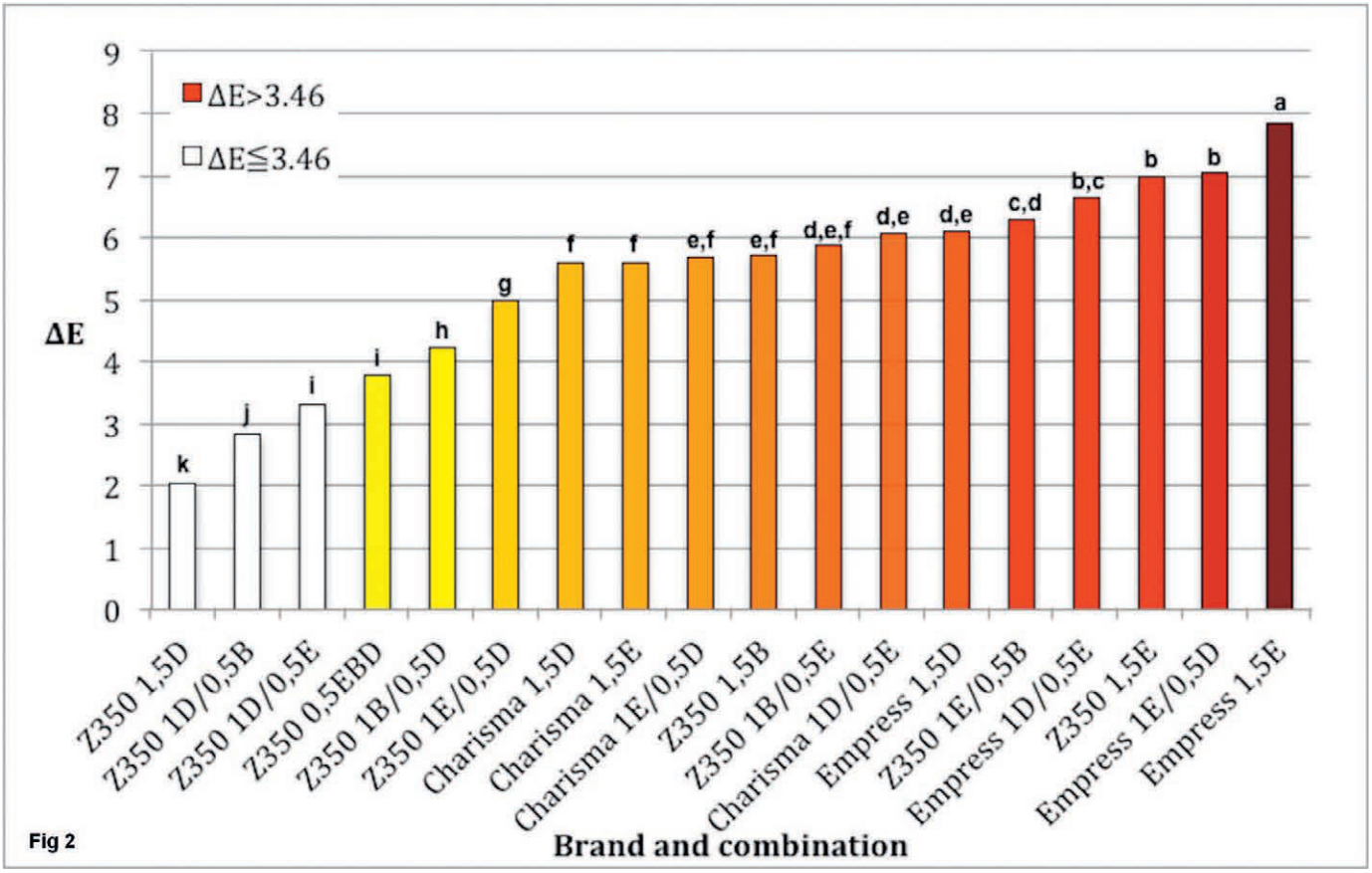

Figure 2. *Mean ΔE^*_{ab} values graph of the resin composite combinations. White color bars ($\Delta E^* \leqq 3.46$) indicate clinically acceptable values. Darker colors are related to lower masking ability. Groups that do not share a letter are statistically different.*

Diamond systems were capable of masking the C4 background. The null hypothesis was totally rejected, because the masking ability was different among tested combinations and resin composite restoration systems. In this sense, the masking ability of the resin composites may be affected by the formulation components of the material, such as opacifiers, pigments, and fillers.[4] The fact that only the Z350 XT shade combinations were considered clinically acceptable against C4 background could be explained by the noticeable formulation difference observed through the restoration systems, especially by the filler particles size, composition, and monomer composition.[11,19-22] Thus, ideally, a resin composite restoration system for any clinical situation should balance these composition properties aforementioned to provide either translucent or opaque shades for an adequate layering technique.

The reference used for ΔE^*_{ab} and ΔE_{00} calculation was the color measurement of the specimens of each group positioned over the representative inherent color of the resin composite system. The final measurement used for the color differences calculation was the color of the specimens for each group, positioned over the C4 ceramic background. For discoloration analysis, ΔE^*_{ab} values above 3.46 and ΔE_{00} above 2.25 were considered clinically unacceptable, because color shifts over this threshold value might not be considered acceptable in more than 50% of the time.[17,18] The sample size of this study (n=5) was established based on color measurement standards and previous studies that assessed color differences on resin-based specimens.[7,22,23] Although this study evaluated only resin composite restoration systems, other restorative materials, such as ceramics, could be assessed through this method regarding its masking ability.

Important differences were observed between CIELAB and CIEDE2000 results in the color difference calculation. The masking performance of Charisma Diamond and Empress Direct dentin SL groups were statistically similar through

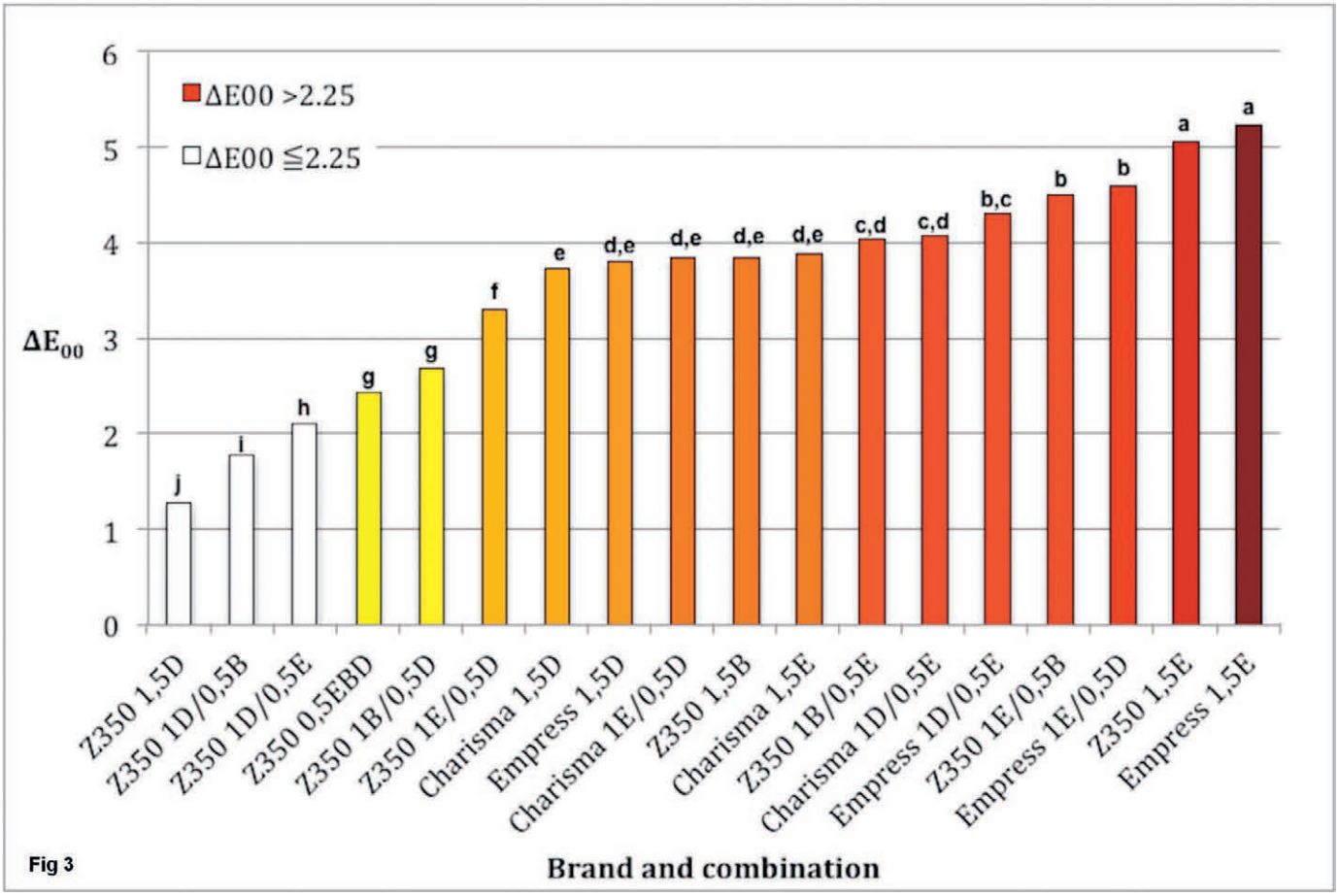

Figure 3. *Mean ΔE_{00} values graph of the resin composite combinations. Groups with white color bars ($\Delta E_{00} \leqq 2.25$) indicate clinically acceptable values. Groups that do not share a letter are statistically different. Same letter indicates similar masking performance.*

CIEDE2000 analysis but different at CIELAB calculation. For Z350 XT and Empress Direct enamel SL groups, the same pattern was observed: similar masking ability when using the CIEDE2000; however, a statistically significant difference was observed through CIELAB. Also, a similar masking ability among SL groups Empress Direct 1.5D, Charisma Diamond 1.5D and 1.5E, and Z350 XT 1.5B could be observed for CIEDE2000 but not for CIELAB. These discrepancies in results can be explained by the differences between equations. Although CIELAB calculates the total color difference, with equal weight for all color coordinates in the formula, CIEDE2000 makes important adjustments that approximates the analysis in a manner similar on how the human eye perceives the color differences. The weighting functions (S_C, S_H, and S_L) adjust the weight of color coordinates related to chroma, hue, and lightness in the total ΔE value. In addition, the rotation function (R_T) weights the interaction between hue and chroma differences in the blue region of visible color spectrum.[15,18,24] These adjustments may result in an adequate fit with the visual judgments[18] and could produce numerical differences in ΔE values and thresholds.

The layering technique for resin composite restorations is often used to reduce shrinkage stress on the adhesive interface and for better tooth shade matching.[25] Physical properties of the resin composite, such as light transmission, dispersion, and reflection, can be affected by the layering technique, because each layer independently applied leaves an organically rich zone on the surface, affecting light transmission by enhancing light dispersion and diffusion.[25] Consequently, this technique reduces the translucency of the restoration and minimizes the interference of background color.[25] In the present study, the combination of 1.0 dentin shade and 0.5 body

shade of the Z350 XT system showed the lowest CIELAB (ΔE^*_{ab}=2.83) and CIEDE2000 (ΔE_{00}=1.77) color differences values among multilayered groups ($p<0.05$). Likewise, the Z350 1.0D/0.5E group presented clinically acceptable values for CIELAB (ΔE^*_{ab}=3.30) and CIEDE2000 (ΔE_{00}=2.12) color difference calculation. The layering technique with 1.0 mm Z350 XT dentin + 0.5 mm of any relatively more translucent composite (body or enamel shades) resulted in lower ΔE values, in comparison with the same shade combination with a thicker translucent layer (1.0 mm enamel shade). These results suggest that esthetic restorations capable of masking extreme discolored background can be achieved by correct layering shade combinations if the adequate opaque-translucent proportion is applied: a thinner enamel shade layer with a thicker opaque shade layer.[26] Friebel and others stated that combinations between thinner layers of enamel shades and thicker layers of dentin shades promote more harmonic restorations.[26] As demonstrated in the present study, this resin composite shade combination pattern can also be applied over discolored teeth and is capable to mask the color discrepancies.

Masking discolored background requires minimal opaque-shade thickness, which depends on the resin composite brand, composition, and translucency.[4,7] Three combinations in the present study were considered clinically acceptable by both color difference analysis. All those groups commonly had the Z350 XT dentin shade, with thickness of 1.0 mm combined with body and enamel shades or with 1.5 mm thickness in the SL group. The latter also presented the lowest ΔE^*_{ab} and ΔE_{00} values among the groups. Kim and others[7] and An and others[4] pointed out that different resin composite opaque shades require a minimal thickness to mask darker backgrounds. In the present study, 1.0 mm Z350 XT dentin shade was necessary to mask the C4 background.[4,7] This finding is supported by An and others,[4] who established that an opaque shade thickness ranging from 0.8 to 1.45 mm has to be used to mask a C4 shade background.

The Z350 XT dentin shade can be considered less susceptible to color background interference than the dentin shades of the other two systems. Both Empress Direct and Charisma Diamond dentin shade composites had ΔE^*_{ab} values over 3.46 and ΔE_{00} values over 2.25, even with 1.5 mm in thickness. An and others[4] reported that a correlation may exist between translucency and masking ability and that both are affected by the brand and the shade of the resin composite. These findings are in agreement with the present study, where Filtek Z350 XT was the only system capable of masking the discolored background. Empress and Charisma composite systems could not mask a C4 background, not even when only a dentin shade was applied over it.

The Filtek Z350 XT restoration system is composed of three different translucencies: enamel, body, and dentin. The handling of this system is considered complex in comparison with the other restoration systems. Proper knowledge about the optical properties (color and translucency) is essential for an adequate restoration.[7] The CIEDE2000 analysis showed that there were no statistically significant differences between the SL Z350 XT body shade group and the SL dentin shade of the Charisma and Empress groups. This finding suggests that the Z350 XT body shade behaves similarly to the dentin shades of the other restorative systems, especially in situations where the background color is an important issue.

SL and multilayer combinations of Charisma Diamond and Empress Direct showed insufficient masking ability against a C4 backing. This background color is considered an extreme discoloration; C4 is the darkest Vitapan Classical shade guide color.[27] The present study addressed masking ability only against a C4 background, but not over other lighter shade backgrounds. Also, only A1 opaque shade composites were used in the present study, without chromatic variations. This does not mean that the same results could also be applied to a less critical discoloration; however, it suggests that both systems should be used carefully in restorations for covering up severely discolored structures. Likewise, the substrate color and available thicknesses should be considered when deciding which composite restoration system should be used. More studies on masking ability against different and lighter backgrounds, varying composite hue and chroma, may be necessary to clarify this issue.

CONCLUSIONS

Based on this study results, the following can be concluded:

1. The resin composite layering technique may be applied on restorations over severely discolored backgrounds.

2. Among the opaque resin composites, Z350 XT dentin shade showed the best masking performance. This composite can be covered by more translucent composites in the layering technique and should be recommended when masking a dark background, especially when little space is available for restoration.
3. The CIEDE2000 formula is recommended as the equation for dental materials color differences calculation, especially for masking ability assessment.
4. Masking ability and color matching alone cannot guarantee an adequate harmonization of the restoration with the natural adjacent teeth. Optical properties like natural enamel and dentin translucency, superficial textures, and restoration form must be considered, especially in esthetic restorations in anterior teeth.

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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Effect of Finishing and Polishing on the Surface Roughness and Gloss of Feldspathic Ceramic for Chairside CAD/CAM Systems

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Clinical Relevance

Comparison among two furnace-based and five manual systems for finishing and polishing feldspathic ceramic for chairside CAD/CAM systems resulted in clinical recommendations for selection of the most appropriate system able to produce enamel-like surface.

SUMMARY

Objectives: To evaluate surface roughness and gloss of feldspathic ceramic blocks for chairside CAD/CAM systems before and after finishing and polishing.

Methods: VITA Mark II ceramic blocks for the CEREC CAD/CAM system were cut perpendicularly in order to obtain a total of 70 specimens (14 × 18 × 3 mm). The flat surface was roughened using a grinder/polisher with dry 120-grit silicone-carbide paper. Surface roughness and gloss were measured using a digital profilometer (Ra) and a glossmeter (GU), respectively. Specimens were randomly divided into seven groups (n=10) based on the finishing/polishing system as follows: 1) Identoflex NGPorcelain Polisher (INP), 2) Identoflex Diamond Ceramic Polisher (IDP), 3) Hiluster Polishing System (HPS), 4) OptraFine (OF), 5) Identoflex Lucent (IL), 6) VITA Akzent Glaze Spray (AGS), and 7) VITA Shading Paste and Liquid (SPL). Surface analysis was repeated after the finishing/polishing treatment, and the obtained data were compared to the baseline in order to evaluate the ΔRa and ΔGU. Results were statistically analyzed. The surface morphology was observed by scanning electron microscopy.

Results: The mean surface roughness of polished systems increased in the order (statistical groups designated) SPLa < ILa < OFab < IDPbc < AGSbc < INPbc < HPSc and mean

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gloss decreased in the order AGSa > SPLa > OFab > ILabc > HPSbcd > INPcd > IDPd.

Conclusions: The smoothest surface of CAD/CAM feldspathic ceramic blocks was achieved using the furnace-based glaze systems VITA Akzent Glaze Spray and VITA Shading Paste and Liquid and manual systems Identoflex Lucent and OptraFine.

INTRODUCTION

The demands for enhanced esthetics of dental restorations have resulted in an increased use of all-ceramic materials.[1] Metal-free restorations were introduced in the 1960s and evolved over the intervening decades. The same is true for the introduction and development of CAD/CAM technology over the past 25 years.[2] These systems have become less expensive, more user friendly, and more precise, thus improving the clinical performance of all-ceramic restorations.[3] Several CAD/CAM systems are currently available on the market. Among them, some consider the CEREC system (Sirona, Bernsheim, Germany) the reference system for the "chairside" procedure, in which the prosthetic restoration is produced entirely by the dentist in the office during a single appointment.[4] In accordance with clinicians' demands, other CAD/CAM systems for chairside procedures have been recently introduced, including Planmeca Planscan/Planmill (Planmeca Oy, Helsinki, Finland), KaVo ARCTICA (KaVo Dental GmbH, Biberach, Germany), and Carestream CS solutions (Carestream Dental, Atlanta, GA). CAD/CAM materials allow combining the advantages of metal-free restorations, such as biocompatibility, durability, and esthetics,[5] with the advantages of computer-aided technology, including the reduction of clinical steps, shorter fabrication time, and lower cost.[4] While specific procedures, such as crystallization, glass infiltration, or sintering, are needed for some ceramic materials, feldspathic ceramic (with or without leucite) does not require additional laboratory processing after milling and is fully compatible with chairside procedures. However, milling feldspathic blocks does not produce ready-to-cement restorations. The milling process produces rough surfaces that require finishing and polishing of the clinically exposed surface. Achieving a smooth ceramic surface is important for several reasons, including esthetics, patient comfort, and biological aspects.[6,7] Staining and plaque accumulation are more pronounced on rough surfaces,[8] thus increasing the likelihood for gingivitis or tooth decay. In addition, rough ceramic restorations are abrasive and can cause greater wear of antagonist teeth.[9] Roughness can also affect the strength of brittle ceramics, thereby causing cracking, chipping, and fracture.[10] Traditionally, finishing and polishing are performed by furnace glazing for feldspathic ceramics fused to metal. When it comes to chairside fabricated restorations, manual finishing and polishing with in-office instruments is a desirable option due to its speed and ease of use. Some studies have reported that manual finishing and polishing systems provided smooth surfaces of layered feldspathic porcelain fused to metal.[11] Less information is available for CAD/CAM blocks. One manufacturer claimed that, due to the industrial sintering process, the CAD/CAM blocks showed a less porous structure of the feldspathic ceramic than for those ceramics that underwent laboratory-based sintering.[12] Manufacturers have also claimed this less porous structure to be a primary reason for the more effective manual polishability of feldspathic CAD/CAM blocks.

As manufacturers consider glaze firing not mandatory but rather as an alternative to manual finishing and polishing, it was considered appropriate to compare the efficacy of different manual finishing and polishing systems and two furnace-based glaze systems on the surface roughness and gloss of feldspathic CAD/CAM ceramic. The null hypothesis was that there was no statistically significant difference in average roughness (Ra) and gloss (GU) among the tested finishing and polishing systems.

METHODS AND MATERIALS

Twenty-five VITA Mark II ceramic blocks for the CEREC CAD/CAM system (VITA Zahnfabrik, Bad Säckingen, Germany) were used. The blocks were cut perpendicularly using a low-speed diamond saw (IsoMet Low Speed Saw, Buehler, Lake Bluff, IL) to obtain 14 × 18 × 3 mm specimens. A total of 70 specimens were prepared. The surface roughness (Ra, the arithmetic average of the profile ordinates within the measured section – average height)[13] of 10 crowns produced using the MC-XL InLab milling unit (Sirona, Bernsheim, Germany) and VITA Mark II CAD/CAM blocks (VITA Zahnfabrik) was measured using a digital profilometer (Mitutoyo SJ-201P, Mitutoyo Corp, Kanagawa, Japan), while a Gloss Meter (Novo-Curve, Rhopoint Instruments, UK) was used for gloss measurements (GU, gloss units). The baseline Ra and GU values, corresponding to the ceramic surface after milling, were 1.08 ± 0.18 μm and 2.37 ± 0.86 GU, respectively. In order

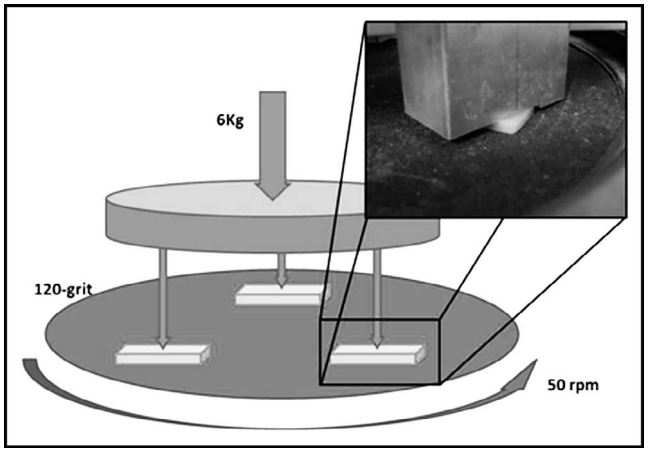

Figure 1. *Roughening scheme and specimen holder.*

to produce the baseline roughness and gloss, specimens were processed with a grinder/polisher (EXTEC Labpol 8, Extec, Enfield, CT) with an equal force distribution during the roughening procedure (Figure 1) using a 120-grit silicon-carbide paper (South Bay Technology, San Clemente, CA) and a 6 kg weight applied on a custom-made support for five seconds at 50 rpm. A new silicone-carbide paper was used for each roughening cycle.

After roughening, the specimens were ultrasonically cleaned in distilled water for 10 minutes before performing the measurements.

The roughness evaluation settings for the profilometer were set with a cutoff value of 0.8 mm, a stylus speed of 0.5 mm/s, and a tracking length of 5.0 mm.[14] Specimens were marked with numbers, and the baseline Ra (Ra^{bl}) was recorded for each specimen. The overall mean specimen Ra was 1.04 $\pm$ 0.25 μm. Gloss assessment was performed at a 60-degree angle following the ISO 2813 specifications for ceramic materials.[15] An opaque silicon mold was placed over the specimens while performing the measurements to eliminate the influence of ambient light and to standardize the reading area of the sample. The baseline gloss (GU^{bl}) was recorded for every specimen; the sample mean baseline gloss was 2.2 $\pm$ 0.6 GU.

The specimens were then randomly divided into seven groups (n=10), as seven different procedures for finishing and polishing were tested. Materials and procedures are reported in Table 1. Manual finishing and polishing procedures were performed by the same operator. The operator was calibrated using a precision scale before and during the procedure, considering that a 40 g force was considered light pressure. The operator calibration was repeated every 10 specimens.[16] Specimens were processed for 30 seconds for each single step. The "small point" polishing point was selected for systems with various tip shapes.

For groups 1 (Identoflex NG-Porcelain Polisher [INP]), 2 (Identoflex Diamond Ceramic Polisher [IDP]), and 3 (HiLuster Polishing System [HPS]), rubber diamond points were used in a low-speed angled hand piece (Kavo INTRAmatic 20CN, Kavo Dental) at 5000 rpm following the manufacturers' instructions. Wet and dry use was implemented as indicated by the manufacturers. The dry condition was chosen because it was considered easier and no

Table 1: *Finishing/Polishing Systems Evaluated*

Groups	Finishing and Polishing System (Code)	Manufacturer	Type	Passages	Batch
1	Identoflex NG-Porcelain Polisher (INP)	KerrHawe SA, Bioggio, Switzerland	Office	Prepolisher—green Gloss—gray High gloss—pink	3186071 3175840 31171192
2	Identoflex Diamond Ceramic Polisher (IDP)	KerrHawe SA	Office	Two-zone technology minipoint—green	3164499
3	HiLuster Polishing System (HPS)	KerrHawe SA	Office	Gloss polishers—pink Diamond polishers—light blue	3066755
4	Optra-Fine (OF)	Ivoclar Vivadent, Schaan, Liechtenstein	Office	Finisher F—light blue Polisher P—dark blue High gloss—diamond paste	PL 1781 PL 1782
5	Identoflex Lucent (IL)	KerrHawe SA	Lab	PVE—green PGR—gray high PVI—pink	124LU
6	VITA Akzent Glaze Spray (AGS)	VITA Zahnfabrik, Bad Säckingen, Germany	Lab	Ceramic spray	21790
7	VITA Shading Paste and Liquid (SPL)	VITA Zahnfabrik	Lab	Shading paste glaze Shading paste liquid	19520 21110

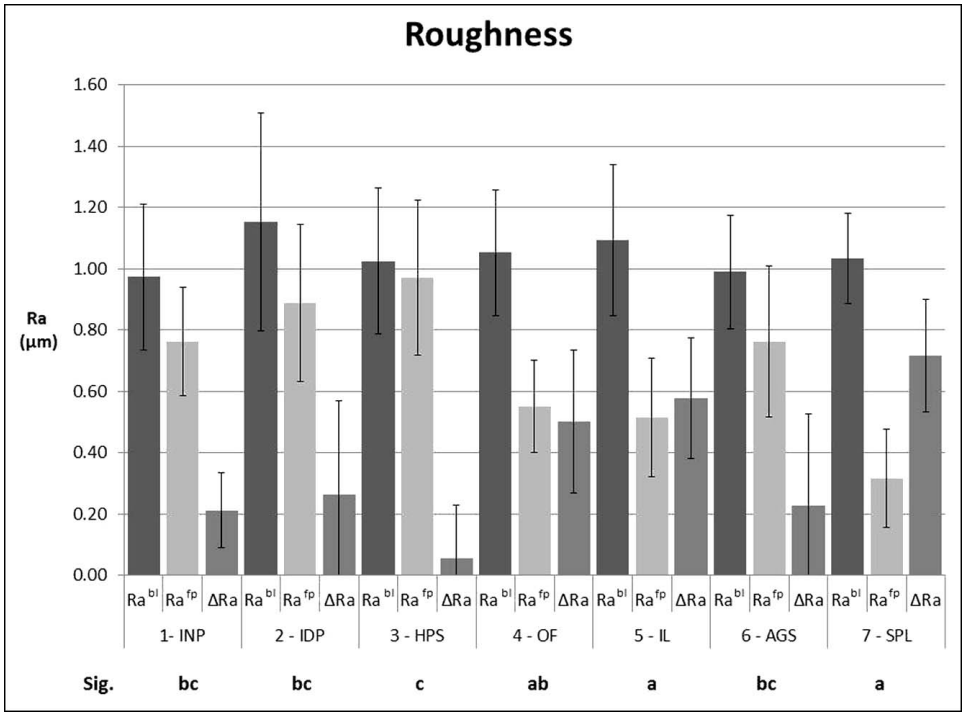

Figure 2. *Bar chart of roughness result.*

risk of tissue overheating is associated with finishing and polishing of a chairside-produced restoration as the procedure is performed extraorally. For group 4 (Optra-Fine [OF]), the rubber diamond points of the first two steps were used at 15,000 rpm and under water cooling in the same low-speed angled hand piece as for groups 1, 2, and 3. The third step, high-gloss polishing with diamond paste, was performed with the same hand piece at 10,000 rpm under wet conditions (paste wetted but no water cooling). For group 5 (Identoflex Lucent [IL]), as the rubber diamond points of the system are marketed only in the "long" mandrel mount, they were used in a straight hand piece (Kavo INTRAmatic 10CH, Kavo Dental) at 15,000 rpm in dry conditions. The procedures for the two furnace-based systems, groups 6 (VITA Akzent Glaze Spray [AGS]) and 7 (VITA Shading Paste and Liquid [SPL]), were performed following the manufacturers' instructions. The firing was performed in the VITA Vacumat 6000M furnace (VITA Zahnfabrik) for both systems.

On completion of the finishing and polishing steps for all seven groups, roughness and gloss measurements were repeated (Ra^{fp} and GU^{fp}), and the obtained values were compared to the baseline. The ΔRa (μm) and the ΔGU were then calculated for each specimen ($\Delta Ra = Ra^{bl} - Ra^{fp}$) ($\Delta GU = GU^{bl} - GU^{fp}$). For roughness, since pooled data for all finishing systems passed the normality test ($p>0.05$), the results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey *t*-tests ($\alpha=0.05$) to determine the level of significance between groups. As pooled data for gloss from all the finishing systems did not pass the normality test ($p>0.05$), the gloss results were analyzed using the Kruskal-Wallis one-way ANOVA on ranks followed by Dunn *t*-tests ($\alpha=0.05$) to determine the level of significance between groups.

Two finished and polished specimens per group were randomly selected, and two extra baseline specimens were processed for observation by scanning electron microscopy (SEM) (JSM-6060LV, JEOL, Tokyo, Japan) at 100× and 1000× magnification to visualize the surface morphology.

RESULTS

Surface Roughness

Means and standard deviations for surface roughness at baseline and after finishing and polishing, difference in roughness, and statistical significance are presented in Figure 2. Significant differences were found between the finishing systems in their ability to reduce after-milling surface roughness of feldspathic ceramic CAD/CAM blocks ($p\leq0.001$). The lowest final Ra value (highest smoothness) was achieved by SPL, but no significant differences were

Table 2: *Means and Standard Deviations (SD) for Surface Roughness and Gloss at Baseline ($Ra^{bl} - GU^{bl}$) and After Finishing and Polishing ($Ra^{fp} - GU^{fp}$), Differences ($\Delta > Ra - \Delta GU$), and Statistical Significance (Different Letters Indicate Statistically Different Groups)*

	1: INP			2: IDP			3: HPS			4: OF			5: IL			6: AGS			7: SPL		
	Ra^{bl}	Ra^{fp}	ΔRa	Ra^{bl}	Ra^{fp}	ΔRa	Ra^{bl}	Ra^{fp}	ΔRa	Ra^{bl}	Ra^{fp}	ΔRa	Ra^{bl}	Ra^{fp}	ΔRa	Ra^{bl}	Ra^{fp}	ΔRa	Ra^{bl}	Ra^{fp}	ΔRa
Mean	0.97	0.76	0.21	1.15	0.89	0.27	1.03	0.97	0.06	1.05	0.55	0.50	1.09	0.52	0.58	0.99	0.76	0.23	1.03	0.32	0.72
SD	0.24	0.18	0.12	0.35	0.26	0.30	0.24	0.25	0.17	0.21	0.15	0.23	0.25	0.19	0.20	0.19	0.25	0.30	0.15	0.16	0.18
Significance	BC			BC			C			AB			A			BC			A		
	GU^{bl}	Gu^{fp}	ΔGU	GU^{bl}	Gu^{fp}	ΔGU	GU^{bl}	Gu^{fp}	ΔGU	GU^{bl}	Gu^{fp}	ΔGU	GU^{bl}	Gu^{fp}	ΔGU	GU^{bl}	Gu^{fp}	ΔGU	GU^{bl}	Gu^{fp}	ΔGU
Mean	2.5	7.0	4.5	1.8	4.9	3.0	2.0	14.6	12.6	2.2	38.7	36.4	2.4	27.9	25.5	2.2	60.7	58.5	2.5	54.7	52.1
SD	0.9	1.3	1.2	0.3	0.6	0.5	0.5	3.9	3.5	0.4	8.8	8.7	0.6	7.6	7.5	0.3	13.6	13.7	0.4	17.4	17.6
Significance	CD			D			BCD			AB			ABC			A			A		

Abbreviations: INP, Identoflex NG-Porcelain Polisher; IDP, Identoflex Diamond Ceramic Polisher; HPS, HiLuster Polishing System; OP, Optra-Fine; IL, Identoflex Lucent; AGS, Vita Akzent Glaze Spray; SPL, Vita Shading Paste and Liquid.

found among the SPL, OF, and IL groups ($p<0.05$). HPS showed the lowest polishing ability, but no statistically significant differences were found among the HPS, IDP, AGS, and INP groups (Table 2).

Gloss

There were no statistically significant differences between the laboratory systems SPL, IL, or AGS and an in-office water-based system, such as OF (Table 2). The lowest ΔGU value was obtained with IDP, and no statistical differences were found with the HPS and INP groups; the ΔGU ranged from 3.0 for IDP to 12.6 for HPS (Figure 3).

SEM Observations

The tested finishing procedures influenced the surface morphology compared to baseline. Figure 4 shows the SEM images of the specimen baseline surfaces and of the tested groups; grooves and surface irregularities were present (Figure 4a). For the manual finishing and polishing systems, the presence of surface defects showed a direct correlation with the final surface roughness and gloss reported in Table 2. The surface treated with HPS exhibited diffuse irregularities and the absence of smooth areas. Conversely, the ceramic surface treated with IL showed an almost complete reduction of grooves present in baseline specimens and

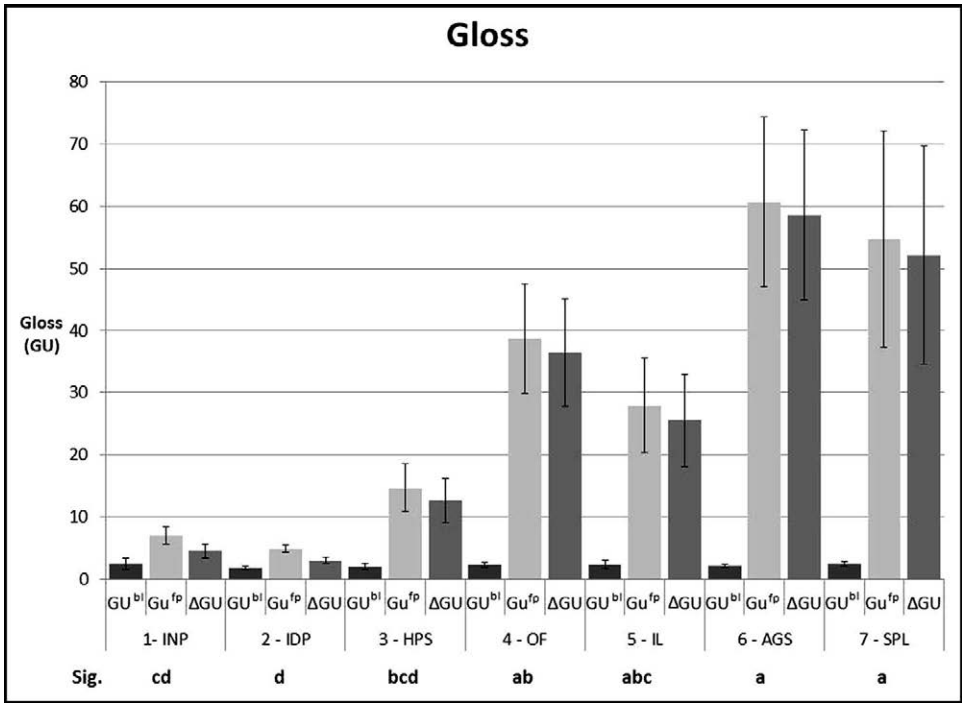

Figure 3. *Bar chart of gloss results.*

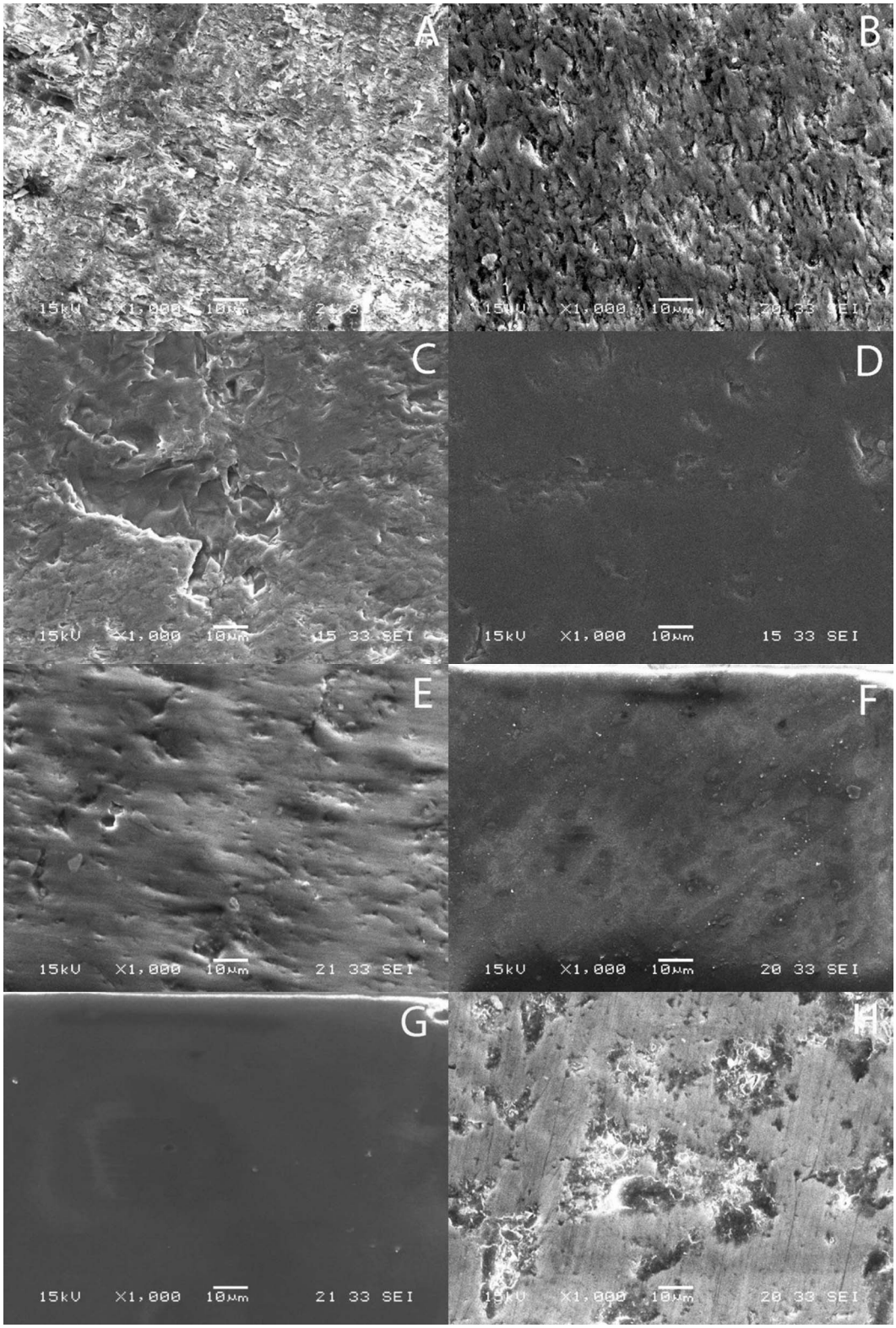

Figure 4. *(A-H): Scanning electron micrograph at 1000× of the different porcelain surfaces analyzed. (A): Baseline specimen. (B): Identoflex NG-Porcelain Polisher (INP). (C): Identoflex Diamond Ceramic Polisher (IDP). (D): HiLuster Polishing System (HPS). (E): Optra-Fine (OP). (F): Identoflex Lucent (IL). (G): Vita Akzent Glaze Spray (AGS). (H): Vita Shading Paste and Liquid (SPL).*

large areas of smooth surfaces. The most regular and smoothest surface was recorded for the two furnace-based glaze systems: SPL and AGS. The SEM images of these two groups showed an absence of grooves and defects. In contrast to the other groups, a poor correlation was found between the SEM appearance and roughness for AGS.

DISCUSSION

The tested finishing and polishing systems exhibited different abilities to smooth the surfaces of Vita Mark II feldspathic ceramic blocks. Therefore, both null hypotheses were rejected.

Roughness and gloss are two important factors for evaluating the surface properties of dental materials after finishing and polishing. Despite a strong correlation between the two parameters,[17-21] they represent two different surface properties. Roughness is a dimensional evaluation of the surface topography that could be described by several linear (Ra, Rq, Rz) or three-dimensional (Sa, Sq, Sz) parameters,[22] while gloss is an optical phenomena that is defined as the property of a surface that involves specular reflection and is responsible for a lustrous or mirror-like appearance.[23]

Even if other systems for the evaluation of surface properties are available, the use of profilometer and Ra measurements is the most common combination for evaluating surface roughness in dentistry. Roughness is a high-frequency, short-wavelength component of a measured surface and refers to the fine irregularity of surfaces, measured in micrometers.[24] The Ra (μm) is the mean value of the distances of the roughness profile to the intermediate height along the measured length.

In order to provide minimal bacterial retention, the average values of surface roughness (Ra) should be lower than 0.2 μm.[25] Ceramics have exhibited the least bacterial and glucan adhesion when compared to other restorative materials.[26] None of the systems tested in the present study were able to achieve an Ra < 0.2 μm. However, it should be noted that Ra values of intact human enamel are generally between 0.45 and 0.65 μm.[27-29] In the present study, SPL, IL, and OF, yielded Ra values similar to those reported for enamel; therefore, they were considered adequate to smooth ceramic surfaces to a clinically adequate level. Conversely, the systems that did not achieve results within this range cannot be considered adequate in terms of clinically acceptable smoothness.

The factors that have been reported to affect gloss include refractive index of the material, angle of incident light, and surface topography.[30] In the present study, the refractive index of the material can be considered constant due to the presence of a single fine ceramic substrate, and the angle of incident light was set to 60 degrees, as indicated by ISO 2813 for specimens of medium gloss.[15] Therefore, the surface topography was considered the main factor influencing the ceramic gloss. In contrast to roughness, a clinically accepted threshold for gloss in terms of GU has not yet been established. Nevertheless, some data are available for enamel surface gloss. When the visual luster of different composites were compared to and approximated natural enamel, the gloss of the latter ranged from 40 to 47 GU.[31] In a more recent study, a value of 53 GU was reported for polished enamel.[32] In the same study, a value of 52 GU was reported for Vita Mark II, comparable to the results in the present study after glazing with SPL.

Although the glazing systems achieved the highest mean ΔGU, there was no statistically significant difference when compared to the intraoral wet system of OF and the laboratory system based on dry rubber tips: IL. This could be explained by the positive influence of the water-cooling operating mode for the OF system and with the peripheral higher speed of the laboratory tips mounted on a straight hand piece when compared to the smaller tips of the other dry intraoral systems tested in the present study. As far as gloss is concerned, only a few systems were able to finish and polish the milled surfaces to the referenced values for enamel (40-47 GU).

Most of the tested manual systems are clinical systems for repolishing feldspathic ceramic surfaces after adjustment, which should be done using fine (red stripe, 40 microns) or extra-fine (yellow stripe, 20 microns) burs. The CEREC milling burs are reported to have a 64-micron grain size, so the initial roughness of the CEREC is higher than the roughness produced by finishing burs indicated for occlusal adjustment. Therefore, the time suggested by the manufacturers of the finishing systems is based on surfaces ground with fine-grained burs and could be insufficient for surfaces milled with the more coarse CEREC milling unit burs in obtaining a clinically acceptable gloss and surface roughness. Vita Mark II feldspathic ceramic has been used in chairside CAD/CAM procedures for more than 20 years and has been evaluated in several studies. In a study on roughness of Vita Mark II ceramic blocks polished using 3M Sof-Lex discs, the reported mean roughness value (Ra=0.46 μm)[33] was comparable to

the best results obtained in the present study. The 3M Sof-Lex system was not tested in the present study, as it cannot be considered a "stand-alone" system for posterior areas. The disc shape does not allow to completely finish/polish posterior restorations, and this system is generally combined with other finishing/polishing components.[34] Another study reported a mean Ra value of 0.6-1.0 μm for Vita Mark II, depending on the finishing system.[35] This is generally in agreement with the present study, although the finishing/polishing systems tested were different. In a study that evaluated the efficacy of diamond pastes combined with other polishing systems, a mean Ra value of 0.4 μm was reported for glazed Vita Mark II,[36] which is similar to the findings of the present study. Other studies also reported that final polishing with a diamond paste did not improve the surface smoothness of ceramic restorations.[37,38] In the present study, OF (the only system that includes a diamond paste) provided satisfactory results, which were in the highest statistical rank with the furnace-based SPL and IL systems. Similar results were reported for the Optra-Fine system and Glazed surface on Vita Mark II ceramic blocks in a study that tested different finishing and polishing systems on two different substrates.[39] Those authors concluded that the same polishing system can yield different Ra values depending on the substrate treated. The good results obtained with OF might also be explained due to its wet use. Another study reported that the best results in finishing and polishing ceramic surfaces were obtained using a fine diamond instrument in a wet condition, with authors indicating that polishing in a dry condition has to be associated with a low rotation speed of the instrument for the best results.[40] One interpretation of these results was that ceramic particles were removed from the surface during the polishing procedure and became part of the abrasive system, thereby enabling lower Ra values. Systems such as IL, intended to be used in dry conditions, resulted in a smooth surface with statistically similar Ra values when compared to a glazed surface.

The SEM analysis highlighted the surface morphology without providing evidence that wet systems produce more homogeneous surfaces when compared to dry systems. A similar surface morphology was observed in the dry system, IL, and in the wet system, OF. The working condition factor (wet/dry) was not analyzed in the current study, and subsequent studies should be performed in order to clarify the influence of these factors for finishing and polishing systems. When INP was compared with other polishing systems, it exhibited a statistically lower ability to smooth a ceramic surface when compared to the glazing system SPL.[41] No data were available on the finishing/polishing effects of AGS, IL, HPS, and IDP, recently introduced as "single-step" ceramic polishing systems. In the present study, the factor "number of steps" could not be directly associated with roughness. INP (three-step system), HPS (two-step system), and IDP (single-step system) did not show statistically significant differences in their mean ΔRa values. It has been noted that the use of a sequence of different polishing discs or tips to achieve the desired smoothness resulted in an increase of polishing time for multistep systems.[1]

One of the advantages of using a CAD/CAM system in association with a chairside material is to produce and cement a ceramic restoration in a single appointment.[42] Time needed for every step of fabrication of chairside restorations is of importance. A report on surface finishing of a dental ceramic at clinically acceptable times (180, 120, 60, and 30 seconds) at every step of multistep polishing systems showed that the use of polishing systems for 60 seconds for each step enabled satisfactory results and reasonable timing.[43] Based on the findings of the present study, where better results were achieved by two manual systems and one furnace-based system, a question arose on clinically preferable timing. One study reported that a glazing procedure would save 20% of operator time when compared with manual polishing.[44] This needs to be considered, even if the time savings is related only to the operator time (and not to patients' time). Indeed, if the restoration is subjected to furnace glazing, the procedure would require a considerable delay of cementation or, more likely, a second appointment. This could save operator time but cannot be considered an effective chairside procedure. Based on the present findings, the polishing/finishing ability of furnace-based and selected manual systems was comparable.

CONCLUSION

Within the limitations of the study, the furnace-based glazing system SPL and the manual, dry, three-step system IL exhibited the best results in terms of roughness even if the difference with the manual, wet, two-step-plus-diamond-paste OF system was not statistically significant. When considering gloss, the two furnace-based systems, AGS and SPL, achieved the best results, even though the differences with the two manual systems, OF and IL,

were not statistically significant. Only two manual and one furnace-based system enabled clinically acceptable smoothness and gloss on an after-milled CEREC surface.

Conflict of Interest

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

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Marginal Adaptation and Quality of Interfaces in Lithium Disilicate Crowns — Influence of Manufacturing and Cementation Techniques

CA Melo Freire • GA Borges • DBM Caldas • RS Santos • SA Ignácio • RF Mazur

Clinical Relevance

More acceptable interfaces can be obtained for lithium disilicate crowns using a self-adhesive resin cement.

SUMMARY

Purpose: To evaluate the cement line thickness and the interface quality in milled or injected lithium disilicate ceramic restorations and their influence on marginal adaptation using different cement types and different adhesive cementation techniques.

Methods and Materials: Sixty-four bovine teeth were prepared for full crown restoration (7.0±0.5 mm in height, 8.0 mm in cervical diameter, and 4.2 mm in incisal diameter) and were divided into two groups: CAD/CAM automation technology, IPS e.max CAD (CAD), and isostatic injection by heat technology, IPS e.max Press (PRESS). RelyX ARC (ARC) and RelyX U200 resin cements were used as luting agents in two activation methods: initial self-activation and light pre-activation for one second (tack-cure). Next, the specimens were stored in distilled water at 23°C ± 2°C for 72 hours. The cement line thickness was measured in micrometers, and the interface quality received scores according to the characteristics and sealing aspects. The evaluations were performed with an optical microscope, and scanning electron microscope images were presented to demonstrate the various features

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found in the cement line. For the cement line thickness, data were analyzed with three-way analysis of variance (ANOVA) and the Games-Howell test (α=0.05). For the variable interface quality, the data were analyzed with the Mann-Whitney *U*-test, the Kruskal-Wallis test, and multiple comparisons nonparametric Dunn test (α=0.05).

Results: The ANOVA presented statistical differences among the ceramic restoration manufacturing methods as well as a significant interaction between the manufacturing methods and types of cement ($p<0.05$). The U200 presented lower cement line thickness values when compared to the ARC with both cementation techniques ($p<0.05$). With regard to the interface quality, the Mann-Whitney *U*-test and the Kruskal-Wallis test demonstrated statistical differences between the ceramic restoration manufacturing methods and cementation techniques. The PRESS ceramics obtained lower scores than did the CAD ceramics when using ARC cement ($p<0.05$).

Conclusions: Milled restorations cemented with self-adhesive resin cement resulted in a thinner cement line that is statistically different from that of CAD or pressed ceramics cemented with resin cement with adhesive application. No difference between one-second tack-cure and self-activation was noted.

INTRODUCTION

Ceramic restorations are aimed at the esthetic, structural, and biomechanical recovery of dental elements. These materials constitute one of the main indirect alternative restorations, as they present favorable properties, such as chemical stability, biocompatibility, high resistance to compression, a thermal expansion coefficient near the dental structure, and optical properties that are similar to the dental tissues, making adequate esthetic and functional results possible.[1-7]

Resistance to fracture and marginal adaptation are among the key factors necessary for longevity and clinical success in indirect restorations.[1-12] These materials can be classified into three groups: the siliceous, the aluminum-based, and the zirconia-based groups.[2,13]

In the siliceous group, lithium disilicate ceramics are widely used, as they contain favorable properties of high intrinsic resistance and adherence.[6,7,13,14] These ceramics are processed using two methods: automation technology using the CAD/CAM system or isostatic injection by heat technology.

The composition of siliceous ceramics includes a silicon dioxide network that makes conditioning possible by means of hydrofluoric acid and the application of silane as a binding agent.[13-15] The internal surface of the conditioned restoration is predisposed to the micromechanical and chemical interaction with the luting agent.[13-15] In turn, silane is a bifunctional molecule with hydrolyzable monovalents that allow for primary chemical bonding between the inorganic materials of the ceramic (SiO_2) and the organic portion of the resin material within its double bonding between carbons.[13,14]

Resin cements promote adhesive continuity between the tooth and the ceramic restoration, using a high content of silicon dioxide, promoting the sealing of the tooth/restoration interface.[6,9,14-17] Such cementing agents can be classified according to the types of applied adhesion strategy,[18] which include the conventional resin cements, used with etch-and-rinse adhesive systems; self-conditioning resin cements, associated with self-etch adhesive systems; and self-adhesive resin cements.[18] This last type requires fewer steps during the cementation process and foregoes the need to treat the dentinal substrate separately, thereby reducing the work time and diminishing the sensitivity of the technique.[7,16,19]

Since the indirect restorations present an interface between the restoration and the dental structure, the vertical thickness of the cement line becomes a determining factor in establishing favorable characteristics of marginal adaptation.[8-12] A wide line of a cementing agent exposed to the oral environment may result in periodontal problems and marginal staining, among other complications.[20,21] To minimize these complications, the adhesive cementation techniques must follow an optimized and rational protocol that seeks to achieve predictable results.[7,14,19,22] This process is influenced by such factors as the type of ceramic, the type of resin cement, the preparation and the appropriate cleaning of the dental substrate, the handling of the material, the activation of the adhesive/cement system, and the method used to remove the excess cement from the margins.[14,19,22] Therefore, procedures that establish the moment and method of removing the excess cement are necessary in order to obtain the characteristics of complete sealing and quality of interface.[22,23]

Within this context, studies[8-12,22-29] show that factors such as the ceramic restoration manufactur-

ing method, the configuration of the crown margin, the space required for the cement, the type of cement, and the cementation technique used can all lead to different results in terms of marginal adaptation.

The present study aims to evaluate the cement line thickness and the interface quality in milled or injected lithium disilicate ceramic restorations and their influence on marginal adaptation using different cement types and different adhesive cementation techniques. The null hypotheses included the following: no differences would be detected between the manufacturing methods for ceramic restorations, between the cements themselves, or between the cementation techniques.

METHODS AND MATERIALS

The present study used 64 lower bovine incisors, which were stored in a 5% Chloramine-T solution. The teeth were cleaned using curettes and a prophylaxis brush with pumice and without fluoride (Pasta Prophy Zircate, Dentsply, Milford, CT, USA).

One base structure was created to fix the teeth, allowing for greater stability of the structures during optical microscopic analysis: mechanical retention was created in the roots prior to the inclusion of chemically activated acrylic resin (Jet, Clássico Dental Producs, São Paulo, SP, Brazil) in polyvinyl chloride (PVC) tubes (Tigre, Joinville, SC, Brazil). The teeth were placed with the long axis parallel to the height of the tube, with the cementoenamel junction positioned approximately 3 mm above the acrylic resin surface.

For the prosthetic preparation of the full crown, the specimens were attached to a lathe (Nardini–ND 250 BE, São Paulo, SP, Brazil) and were prepared under constant water cooling. The final dimensions of the preparations were 7.0 ± 0.5 mm in height, 8.0 ± 0.5 mm in cervical diameter, and 4.2 ± 0.5 mm in incisal diameter. For the preparations, this study used a coarse round end taper diamond bur number 5850.314.018 (Brasseler, Savannah, GA, USA) with 0.8-mm depth at the end line. The diamond bur was replaced after every five preparations. Next, the preparations were finished using fine round end taper diamond burs, numbers 4137 F and 4137 FF (KG Sorensen, Cotia, SP, Brazil) (Fig. 1A and 1B). All angles were rounded, and the preparation finish line was located 1.0 ± 0.2 mm above the cementoenamel junction.

The specimens were divided randomly into two groups according to the manufacturing technique of

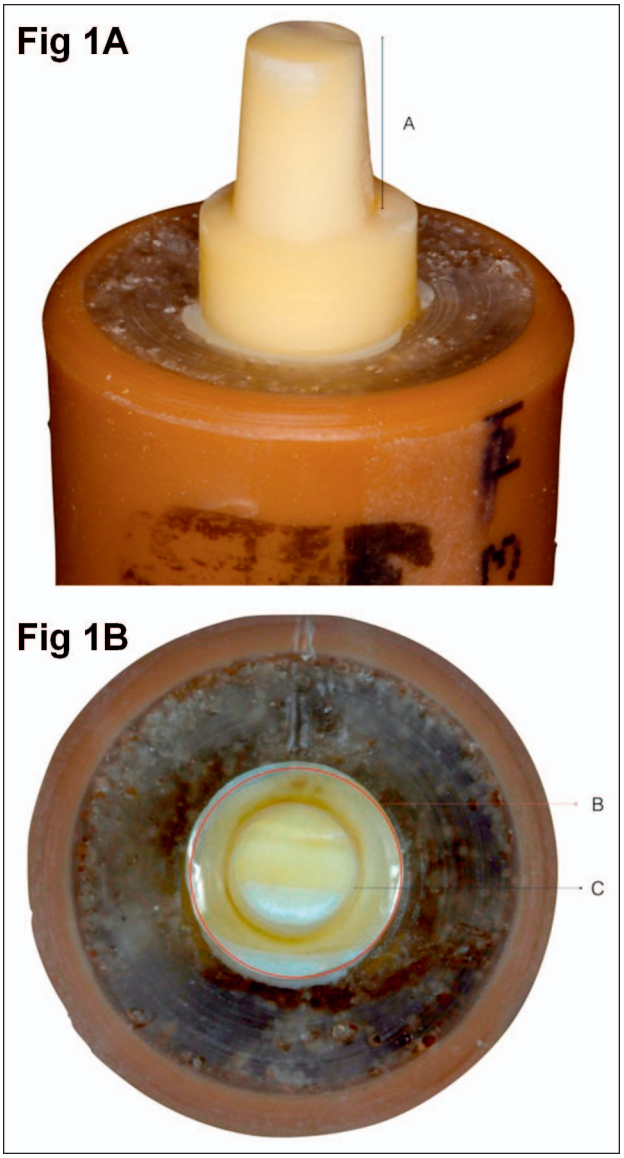
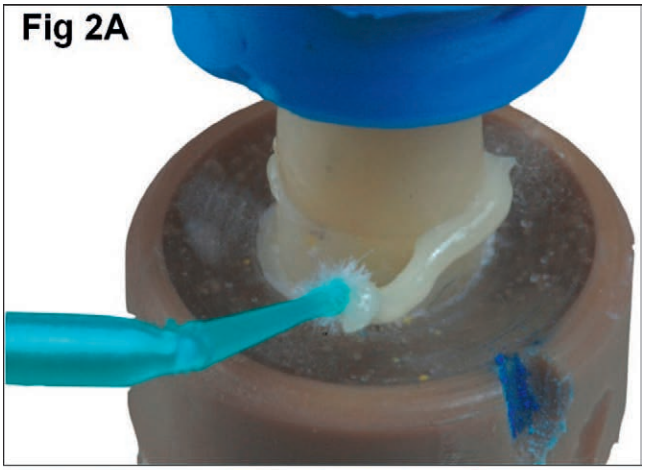


Figure 1. *(A and B) - The final dimensions of the preparations were (A) 7.0 ± 0.5 mm in height, (B), 8.0 ± 0.5 mm in cervical diameter, and (C) 4.2 ± 0.5 mm in incisal diameter.*

indirect restorations made of lithium disilicate ceramics, which was performed by means of the automation technique guided by the CAD/CAM system (IPS e.max CAD, Ivoclar Vivadent, Schaan, Liechtenstein) or isostatic injection by heat technology (IPS e.max Press, Ivoclar Vivadent).

Two types of resin cements were used to cement the ceramic crowns: dual-activation conventional cement (RelyX ARC, 3M ESPE, St Paul, MN, USA) and dual-activation self-adhesive cement (RelyX U200, 3M ESPE, Seefeld, Germany).

Two techniques were executed to remove the excess cement at the crown margins of the restora-



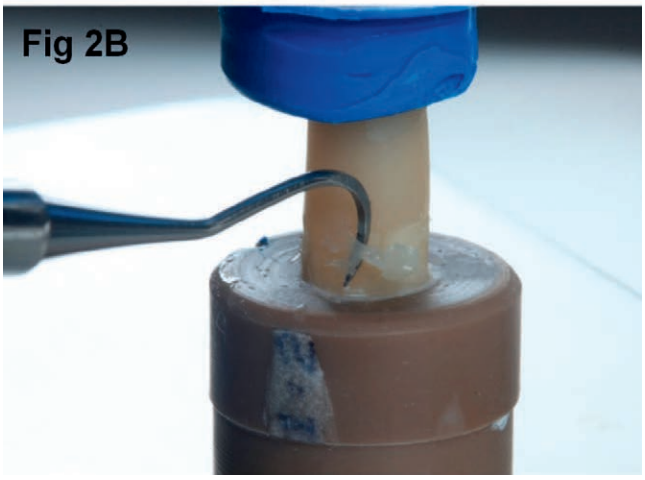


Figure 2. *(A and B) - Removal of the excess cement using the activation tack-cure. (A) Initial removal with application tips; (B) exploration and final removal with curette.*

tion after performing two activation methods: initial chemical activation or initial pre-activation for one second (tack-cure 1s) (Figure 2A and 2B). All of the materials used in this study are described in Table 1.

Preparation of the Lithium Disilicate Crowns—CAD/CAM System

The 32 ceramic crowns were manufactured by scanning each prepared tooth. The teeth received light air spray. Next, a uniform layer of contrast, without excess, was placed (IPS Contrast Spray, Ivoclar Vivadent), and the optical reading of the prepared teeth was performed with a laboratory scanner (InEos Blue, Sirona Dental Systems, Bensheim, Germany), transferred to a computer, and subsequently processed by an appropriate system software (Cerec inLab. SW4 version of application 4.0.2.45144, Sirona), in which a three-dimensional virtual model was created for each of the 32 teeth. Next, the external margin of the tooth and the preparation's finish line were defined, and the adaptation was measured along the entire extension of the crown.

Each IPS e.max CAD ceramic block was placed in the milling unit (In Lab MC XL–102591, Sirona), which had two diamond burs, one cylindrical, with a diameter of 1.2 mm, and the other conical. Next, from the digital model, the blocks were milled to create the crowns. The restorations were completely cleaned, and all of the residues from the milling additive from the CAD/CAM unit were fully removed. For the crystallization process, each crown

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

Material (Manufacturer)	Composition
RelyX ARC (3M ESPE, St Paul, MN, USA), Lot N527302	Bis-GMA, TEGDMA, particles of zirconia/silica, pigments, benzoyl peroxide, amine and photoinitiator
RelyX U200 (3M ESPE, Seefeld, Germany), Lot 510088 (also available as RelyX Unicem 2)	Base paste components: glass powder treated with silane, 2-propenoic acid, 2-methyl,1,1-[1 (hydroxymethyl)-1,2-ethanodiil] ester, TEGDMA silane-treated silica, fiber glass, sodium persulfate and *t*-butyl per-3,5,5-trimethyl hexanoate Catalyst paste components: glass powder treated with silane dimethacrylate caption by substituted dimethacrylate, silane-treated silica, sodium *p*-toluenesulfonate, 1-benzyl-5-phenyl-acid barium salts, calcium, 1,12-dodecane dimethacrylate, calcium hydroxide, and titanium dioxide
Adper Single Bond Plus (3M ESPE, St Paul, USA), Lot N520165	Acid components: phosphoric acid, water, polyvinyl alcohol; primer/adhesive components: ethanol, Bis-GMA–treated colloidal silica, HEMA co-polymer of acrylic acid and itaconic acid, 1,3-glycerol dimethacrylate, water, UDMA, 1,3-glycerol dimethacrylate, EDMAB
RelyX Ceramic Primer (3M ESPE, St Paul, USA), Lot N438638	Ethanol, water and silane methacrylate, 3-trimethoxysilyl propyl methacrylate
IPS e.max CAD and IPS e.max Press (Ivoclar Vivadent, Schaan, Liechtenstein)	Block and tablet ceramic lithium disilicate–based components: SiO_2; additional contents: Li_2O, K_2O, MgO, Al_2O_3, P_2O_5 and other oxides

Abbreviations: Bis-GMA, bisphenol A diglycidyl methacrylate; EDMAB, ethyl-4-(dimethylamino) benzoate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

was positioned on pins for crystallization (IPS e.max CAD Crystallization Pin, Ivoclar Vivadent), and its inner portion was filled with auxiliary firing paste (IPS Object Fix Putty/Flow, Ivoclar Vivadent) to the edge of the restoration margin.

Preparation of the Lithium Disilicate Crowns—PRESS SYSTEM

Thirty-two prepared teeth were impressed with polyvinylsiloxane (Express, 3M ESPE) through the double-impression technique in individual molds manufactured in PVC (Tigre). Type IV plaster (Fuji Rock, GC America, Aslip, IL, USA) was used to manufacture the dies. The teeth were stored in distilled water at 23°C ± 2°C until the cementation process. The dies received a layer of spacer (Spacelaquer Ducera Lay, Degussa Huls, Hanau, Germany) approximately 1 mm above the finish line. The dies were isolated with insulator (Die Lube, Dentaurum J.P. Winkelstroeter KG, Pforzheim, Germany), and wax patterns with 0.7-mm thickness were prepared over the master dies using a wax immersion unit (Hotty, Renfert, Hilzingen, Germany). Next, the wax patterns were included in a coating agglutinated by phosphate from the IPA system itself (PressVest Speed, Ivoclar Vivadent). After the coating was set, a sequence of two heating stages was applied, in which the temperature was increased from 58°C/min to 250°C and maintained for 30 minutes before being increased to 58°C/min to 850°C and maintained for one hour. After the pre-heating stage, the coating cylinders were immediately transferred to the EP500 press oven (Ivoclar AG, Ivoclar Vivadent). The temperature was 920°C and then compression was completed. The coating cylinders were removed from the oven and cooled for two hours. The cooled specimens were removed from the coating using an 80-μm glass bead blasting (Williams Glass Beads, Ivoclar North America, Amherst, NY, USA). The final dimension of the crowns was 7.0 ± 0.5 mm in height and 8.0 ± 0.5 mm in cervical diameter, with a thickness of 2.0 mm, measured with a digital caliper (Mitutoyo, Suzano, SP, Brazil).

Crown Cementation

All of the specimens were cleaned with a bristle brush and pumice without fluoride (Prophy Zircate, Dentsply, Rio de Janeiro, RJ, Brazil) and were washed with a water/air spray.

In the cementations using RelyX ARC, the tooth substrates were treated with an Adper Single Bond Plus adhesive system (3M ESPE). Phosphoric acid at 35% (Ultra-Etch, Ultradent do Brazil Ltda, Indaiatuba, SP, Brazil) was initially applied for 15 seconds on the enamel along the finish line, followed by 15 seconds on dentin, and was then washed for one minute with air/distilled water spray, removing the excess water with high-speed suction, and gently air-dried without desiccation. Next, two layers of the adhesive system were applied actively and the excess was removed with applicator tips (Cavibrush, FGM, Joinville, SC, Brazil), followed by evaporation of the solvent for five seconds. The photoactivation was carried out using a light-curing unit (650 mW/cm^2, LED Bluephase, Ivoclar Vivadent) for 20 seconds.

For RelyX U200, after the teeth were cleaned, 35% phosphoric acid (Ultra-Etch, Ultradent do Brazil Ltda) was only applied to the enamel for 15 seconds, and they were next washed for one minute with air/distilled water spray, the excess water was removed with high-speed suction and then gently air-dried without desiccating. The prepared substrates presented the appearance of a slightly moist dentin surface.

The internal surfaces of the lithium disilicate ceramics were conditioned with 10% hydrofluoric acid[11,30] (Condac Porcelana, FGM Brazil) for 20 seconds, followed by an air/distilled water spray for one minute. Next, 35% phosphoric acid was applied for 30 seconds,[30] followed by air/distilled water spray for one minute. After this step, the crowns were placed in an ultrasound with distilled water for five minutes and dried in oil-free air. The silane agent (RelyX Ceramic Primer, 3M ESPE) was applied, and after five minutes the surface was dried with an air spray for 20 seconds.

Cementation Technique Using RelyX ARC and RelyX U200 with Initial Chemical Activation

The cements were handled and applied in a thin layer to the internal surface of the crowns. After three minutes of initial chemical activation, the excess resin cement was removed using applicator tips (Cavibrush, FGM) and later using a curette (Scaler H6/H7, BISCO Inc, Schaumburg, IL, USA). Next, photopolymerization was performed for 40 seconds, with one activation on each of four crown surfaces for a total of 160 seconds with the light-curing unit, using a "soft start" curing program that began with a low light intensity followed by its gradual increase. The light intensity was monitored with a radiometer (Bluephase Meter, Ivoclar Vivadent). The crowns were maintained in position under a constant 454 g pressure during the entire setting process, during the removal of the excess resin cement, and until photoactivation had been completed.

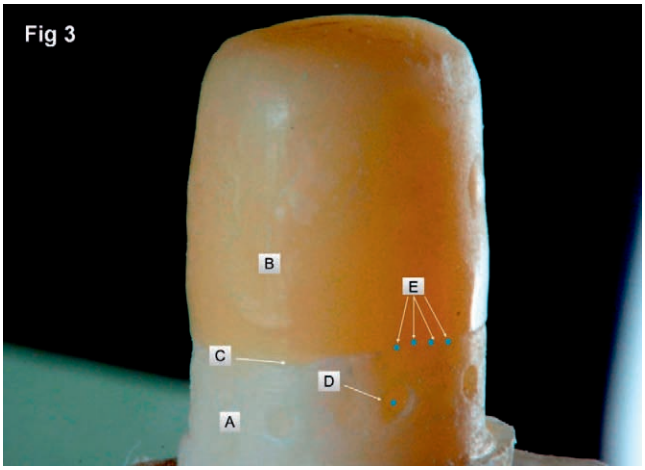


Figure 3. *Illustrative image related to interface quality and measurement locations. (A) Tooth structure, (B) ceramic, (C) tooth/restoration interface, (D) marks for guidance, and (E) locations of measurement.*

Cementation Technique Using RelyX ARC and RelyX U200 with Tack-cure 1s

With the exception of the initial chemical activation process of the resin cement, the cementation procedures were performed as described above. For this technique, the method of previous photoactivation was performed for one second for both resin cements at an intensity of 650 mW/cm^2, in an exposure program at low power at buccal and lingual surfaces, at a distance of 3.0 mm from the activating tip to the full crown, using a light-curing unit. Next, photopolymerization was performed as described above.

Analysis of the Marginal Adaptation

To measure cement line thickness and to conduct the interface quality analysis, eight marks were made on the surfaces of the tooth roots, symmetrically placed 2.0 mm apical to the end of the tooth preparation; the tooth roots had been marked using a number 1011 spherical diamond bur (KG-Sorensen, São Paulo, SP, Brazil). The defined areas served as reference, and between these marks, four measurements were taken, totaling 32 measurements per specimen (Figure 3). The cement line thickness was evaluated through the vertical measurement of the cement line areas between the crown margins and the preparation finish line. To measure the marginal adaptation, an optical microscope was used (STM, Olympus Optical Co Ltda, Tokyo, Japan) at 30× magnification and with the images being displayed by the digital reading unit for coordinates X and Y (MMDC 201, Olympus Optical Co Ltda), with registered values in micrometers and with precision of 0.5 μm.

To analyze the interface quality, the following score values were used according to the cement line characteristics and sealing: 1 = deficient: irregularities were found with discontinuity; 2 = regular: minor irregularities existed but without the presence of discontinuity; and 3 = satisfactory: sealing presented a cohesive pattern, maintaining an integral margin along the entire extension.[23]

Scanning Electron Microscopy

The scanning electron microscope (SEM) micrographs were presented to demonstrate the various features found in the cement line, showing the measurement areas and interface quality. Sixteen crowns were used, eight crowns from each group, and they were subdivided by cement type and cementation method. One impression was taken from the prepared teeth using Express polyvinylsiloxane (3M ESPE) using the double-impression technique in individual molds manufactured in PVC (Tigre), and a replica was created using an epoxy resin material (EpoxiCure 2 Resin, Buehler An ITW Company, Lake Bluff, IL, USA). The epoxy resin models used to evaluate the restoration marginal adaptation were sputter-coated with gold (Balzers-SCD050, Oerlikon Balzers, Balzers, Liechtenstein) for 180 seconds at 40 mA. The images were obtained with a SEM (LEO 435 VP, LEO, Cambridge, UK) at 20 kV with magnification from 50× to 200×.

Statistical Analysis

To evaluate whether there was a difference in the mean values for the variable cement line thickness according to the manufacturing techniques of the ceramic restorations (CAD and PRESS), cements (ARC and U200), and cementing techniques (initial self-activation and the tack-cure), three-way analysis of variance (ANOVA) was applied. When ANOVA indicated a statistically significant difference in the mean values of the dependent variable cement line thickness, according to the combination of the analyzed factors (manufacturing techniques, cements, and cementing techniques), the multiple comparisons post hoc test between the different factor levels was made using the multiple comparisons Games-Howell test for heterogeneous variances, since the Levene test showed heterogeneous variances among the analyzed factors. The significance level for all tests was set at $\alpha = 0.05$. For the variable interface quality that displayed an ordinal scale, the nonparametric Mann-Whitney *U*-test was used when the analysis involved each isolated factor. For treatment interactions, the Kruskal-Wallis test

Table 2: *Average and Standard Deviation of the Cement Line Thickness (in μm) in Relation to the Ceramic Restorations' Manufacturing, Types of Cement, and Cementation Techniques*[a]

Manufacturing Methods	ARC Self-activation	U200 Self-activation	ARC 1s	U200 1s
CAD	62.09 ± 28.79 Aa	52.07 ± 27.97 Bb	61.17 ± 30.42 Aa	52.41 ± 29.66 Bb
PRESS	70.65 ± 71.47 Aa	77.19 ± 78.29 Aa	62.16 ± 51.79 Aa	69.36 ± 74.49 Aa

Abbreviations: ARC, RelyX ARC; CAD, IPS e.max CAD; U200, RelyX U200.
[a] *Different uppercase letters in each row and different lowercase letters in each column represent statistical differences (p<0.05).*

was applied. When the Kruskal-Wallis test indicated differences between the average scores of at least two treatments, comparison between them was made using the multiple comparisons nonparametric Dunn test. The significance level was set at $\alpha = 0.05$. The statistical analysis was performed using the software IBM SPSS Statistics 22.0 (IBM Corp, Armonk, NY, USA).

RESULTS

Cement Line Thickness

The three-way ANOVA test presented statistical differences among ceramic restoration manufacturing methods and a significant interaction between the manufacturing methods and the types of cement ($p<0.05$).

The Games-Howell test demonstrated statistical differences for the CAD with regard to resin cement ($p<0.05$). The U200 resin cement presented lower values of cementation thickness when compared to the ARC resin cement in the CAD technique ($p<0.05$). When compared, the U200 cement for the two methods of CAD and PRESS manufacturing presented statistical differences ($p<0.05$). The lower values of marginal adaptation were obtained for the CAD technique. The average values are presented in Table 2.

Quality of the Interface Cement Line Thickness

The Kruskal-Wallis test and multiple comparisons nonparametric Dunn test presented statistical differences in the cementation techniques when comparing the manufacturing methods for ceramic restorations; the values for PRESS presented lower scores ($p<0.05$). The scores obtained for the cementation quality in the PRESS technique, using preactivation for one second, presented statistical differences ($p<0.05$), showing higher scores both for the ARC cement as well as for the U200 cement, as compared to the conventional cementation technique (initial chemical activation). The means are presented in Table 3.

Correlation Between Cement Line Thickness and Quality of the Interface

The multiple comparison Games-Howell test of heterogenic variances showed that mean values of cement line thickness did not present significant differences between regular and deficient interfaces; however, a satisfactory interface quality presents a mean value of cement line thickness (μm) that is thinner compared to those of regular and deficient interfaces. The comparison is showed in Tables 2 and 3.

SEM

Illustrative SEM images, showing the locations of measurement and the interface quality, are shown in Figures 4 through 7.

DISCUSSION

The interface of indirect restorations can be considered acceptable when it presents cement line thickness values of approximately 120 μm and when the interface quality presents a uniform aspect without the presence of cracks or irregularities.[5,9,23,31,32] According to these requirements, the elements evaluated in this study were within clinically acceptable limits.

Table 3: *Mean and Standard Deviation of Interface Quality for Different Ceramic Restorations' Methods of Manufacturing, Cement Types, and Cementation Techniques*[a]

Manufacturing Methods	Cementation Techniques and Activation Method			
	ARC Self-activation	U200 Self-activation	ARC 1s -	U200 1s
CAD	2.98 ± 0.13 Aa	2.98 ± 0.13 Aa	2.93 ± 0.31 Aa	2.96 ± 0.19 Aa
PRESS	2.82 ± 0.43 Bb	2.90 ± 0.30 Ab	2.94 ± 0.26 Aa	2.90 ± 0.36 Aa

Abbreviations: ARC, RelyX ARC; CAD, IPS e.max CAD; PRESS, IPS e.max Press; U200, RelyX U200.
[a] *Different uppercase letters in each row and different lowercase letters in columns represent statistical differences (p<0.05).*

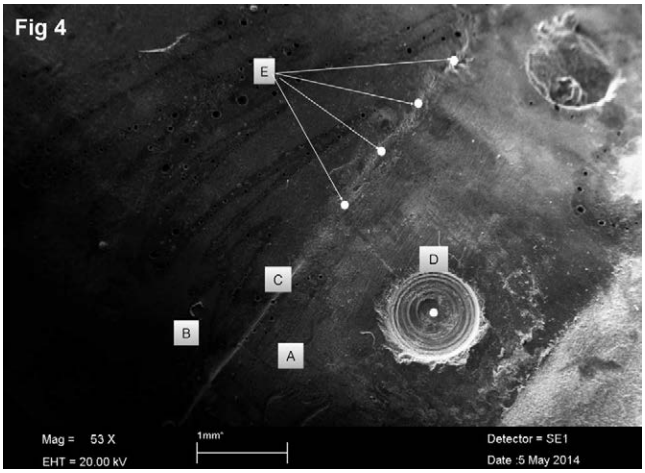


Figure 4. *Sample and MEV image. (A) Tooth structure, (B) ceramic, (C) tooth/restoration interface, (D) marks for guidance, and (E) locations of measurement.*

The suggested null hypotheses were rejected for both the ceramic manufacturing techniques and the types of cement, given that a significant difference in the marginal adaptation could be observed.

The measurement of the vertical thickness of the cement line showed the best adjustment for the CAD crowns when compared to the PRESS crowns. These results corroborate with those of Ng and others,[8] who compared the two methods of manufacturing, pointing out the difficulties associated with the PRESS method. The CAD ceramics required fewer laboratory steps, allowing for a greater control of the work stages.[8,9] By contrast, the PRESS ceramics can undergo a greater influence from independent factors, such as the types of impression materials, the molding technique, the variation in temperature during the transport of the materials to the laboratory, the attainment of the plaster model, the laboratory states for the manufacturing of a piece, and the actions on the part of the operator.[8,9,19]

In the evaluation of the cements, the U200 presented lower thickness values of the cement line when compared to the ARC for the crowns manufactured using the CAD method. This fact may well be related to the stages of adhesion and photoactivation, in which the activation of the adhesive in the dental substrate, according to manufacturer instructions, was carried out prior to the placing of the indirect restorations, which may have caused an increase in the cement line thickness.[7-9] In addition, one must consider the difficulty of limiting oneself to the thickness of the adhesive/cement layer, as this condition can accelerate the mechanism of fatigue and cause early failure.[7,9] These results corroborate with findings from Borges and others,[11] who compared the influence of different types of cements on the marginal discrepancy in pure ceramic systems. Clinically speaking, one must observe aspects such as the viscosity of the cement and its correct manipulation protocol.[9,14,17,27]

The marginal discrepancy of the two methods of cementation evaluated in this study was not affected by the initial chemical activation, nor by the tack-cure 1s, as shown in Table 3. It is suggested that the tack-cure 1s pre-activation method and the removal of its excess, with the aid of a brush and scaler, can lead to a faster and more efficient procedure, which is a clinically relevant consideration. Paying close attention to attainment of the activation time of only one second is highly recommended, since a time of greater than three to five seconds can produce areas of hard resinous cement on the surface, as compared to fluid cement in deeper regions, which can cause maladjustments and dislocations of the fluid and poorly activated cement areas. This type of pre-activation does not affect the intrinsic characteristics of the resinous cement, according to Flury and others,[33] who held that the physical properties of the cements remain unaltered when activation is performed for five seconds, followed by the removal of the excess of cement and the final activation.[22,33] In another study about the techniques for the removal of excess cement, Anami and others[22] determined that the morphology of the restoration margin and the roughness of the interface affect the accumulation of bacterial biofilms, and these authors also obtained favorable results using brushes in the removal of excess resinous cement. This method, as compared to other such removal methods, better prevents against bacterial colonization in the adhesive interface.[22] Other important aspects were reported by Conrad and others[5] who explained that the exposure of a wide band of cementing agent to the oral fluids, as well as to mechanical wear, can lead to the dissolution or modification of the adhesive interface's surface aspect, in addition to allowing the formation of niches that promote the accumulation of bacterial plaque, marginal staining, periodontal problems, and restoration failures.[5,20-22] Other relevant aspects include the final characteristics of prosthetic preparation, such as the conicity of the axial slopes, convergence of the walls, smooth surfaces, transitions, and rounded angles, as they promote the appropriate axis of the restoration's longitudinal insertion, thereby avoiding the formation of hydraulic pressure, favoring a better draining of the cement and an appropriate placing of the

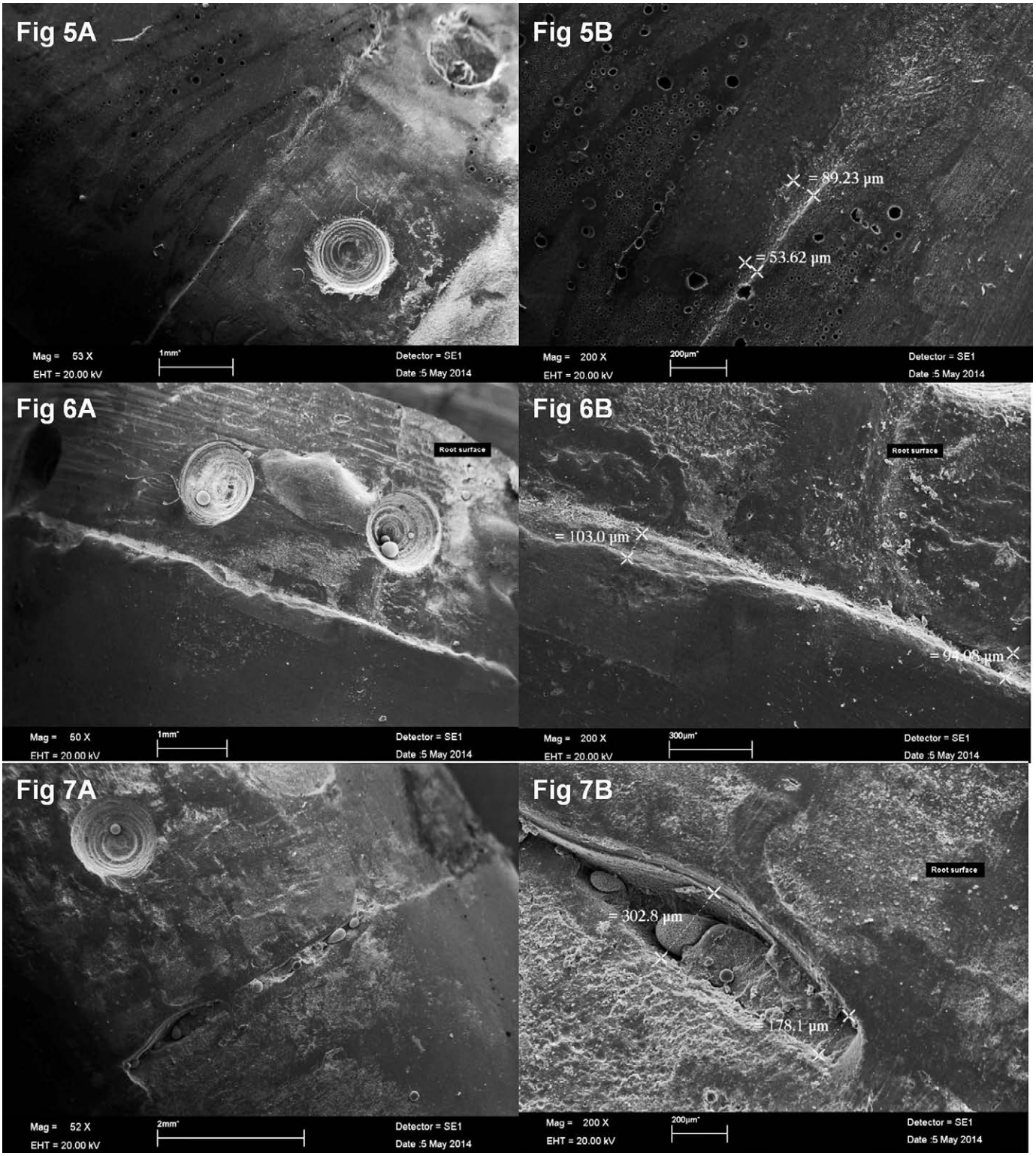


Figure 5. (A and B). *Illustrative image related to interface quality, score 3 = satisfactory, in which case the sealing presents a standard of cohesion and integrated margin along the entire extension. (A) Magnification = 53×; (B) magnification = 200×.*

Figure 6. (A and B). *Illustrative image related to interface quality, score 2 = regular, in which case small irregularities exist but without the presence of discontinuity. (A) Magnification = 50×; (B) magnification = 200×.*

Figure 7. (A and B). *Illustrative image related to interface quality, score 1 = deficient, in which case irregularities were found with discontinuity. (A) Magnification = 52×; (B) magnification = 200×.*

crown itself.[14,17,20,24,25] Some studies[2,7,9,17,18,33-35] have reported that the characteristics found in the preparations for adhesive cementation increase the resistance to fracture (tooth/restoration) by providing a more uniform distribution of strength and a lower concentration of stress. Moreover, the existence of cracks in the cementation line can generate a concentration of stress, which can reduce the restoration's final resistance.[1,3,5,9,17] The tack-cure 1s and the use of a brush to remove the excess cement from the interfaces may also represent an interesting method, and these steps should be followed by a careful light irradiation protocol to obtain the optimal properties of the adhesion and from the resin cement.[17,22,33-35] Given this, further research on this method is warranted, as the resinous cements can present behaviors that are different from their physical-chemical properties.

The interrelationship aspects of the criteria evaluated in this study suggest a greater durability and longevity of the adhesive interfaces, bearing in mind that these factors are limited to the stability and characteristics of each element involved.[4,5,7,9,17,19,22,31,32]

CONCLUSIONS

Within the limitations of this study, it can be concluded that

1. Milled ceramic restorations cemented with self-adhesive resin cement resulted in a thinner cement line compared to the resin cement with adhesive application;
2. No difference between tack-cure 1s and self-activation was noted; and
3. The best interface quality is in fact related to the reduced thickness of the cement line.

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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 Pontifical Catholic University of Paraná, Brazil.

Conflict of Interest

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

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Effect of Bioactive Primers on Bacterial-Induced Secondary Caries at the Tooth-Resin Interface

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C Wu • S-N Chen • GF Pauli • AKB Bedran-Russo

Clinical Relevance

Secondary caries is the primary reason for the replacement of resin composite restorations. Using an artificially induced bacterial carious model, this study found that a bioactive primer containing plant-derived proanthocyanidins inhibited secondary caries formation at the dentin-resin interface.

SUMMARY

Secondary caries at the tooth-resin interface is the primary reason for replacement of resin composite restorations. The tooth-resin interface is formed by the interlocking of resin material with hydroxyapatite crystals in enamel and collagen mesh structure in dentin. Efforts to strengthen the tooth-resin interface have identified chemical agents with dentin collagen cross-linking potential and antimicrobial activities. The purpose of the present study was to assess protective effects of bioactive primer against secondary caries development around enamel and dentin margins of class V restorations, using an in vitro bacterial caries model. Class V composite restorations were prepared on 60 bovine teeth (n=15) with pretreatment of the cavity walls with control buffer solution, an enriched fraction of grape seed extract (e-GSE), 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide/*N*-hydroxysuccinimide, or chlorhexidine digluconate. After incubating specimens in a bacterial model with *Streptococcus mutans* for four days, dentin and enamel were assessed by fluorescence microscopy. Results revealed that only the naturally occurring product, e-GSE, significantly inhibited the development of secondary caries immediately adjacent to the dentin-resin interface, as indicated by the caries inhibition zone. No inhibitory effects were

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observed in enamel margins. The results suggest that the incorporation of e-GSE into components of the adhesive system may inhibit secondary caries and potentially contribute to the protection of highly vulnerable dentin-resin margins.

INTRODUCTION

Resin composite is commonly used for the direct restoration of missing tooth structures. In 2006, the number of resin composite restorations placed in the United States was 121 million, compared with 52.2 million for its alternative, amalgam.[1] In addition to esthetics, resin composites can adhere to tooth structure[2] and allow for a conservative tooth preparation.[3] However, the service life of resin composites is consistently shorter than amalgam.[4,5] A 1%-3% annual failure rate has been reported for resin composite restorations with 50%-75% of failures resulting from secondary caries, followed by postoperative sensitivity and restoration fracture.[2,6-8]

As secondary caries develops at the margins between the restorative material and the tooth substrates, methods to improve the properties of the adhesive interface have been investigated. Resin polymerization reactions are associated with a volumetric shrinkage that induces internal contraction stresses at the interface. The degree of polymerization and shrinkage was positively correlated with interfacial gap size as examined by microtomography.[9] This may lead to marginal breakdown, marginal staining, and possible sites for the development of secondary caries.[10] Furthermore, water absorption into porosities and degradation of resin by esterase activity in saliva contribute to the breakdown of margins over time.[11]

Approaches to strengthen the anchoring dentin matrix have gained increased interest. Specifically, the biomodification of dentin matrix by bioactive agents mediating exogenous collagen cross-linking.[12] Carbodiimide, a synthetic chemical agent, was previously shown to reinforce dentin matrix and stabilize the dentin-resin bond over time by inducing zero-length cross-links.[13] Proanthocyanidins (PACs) are plant-derived polyphenolic compounds derived from catechins and form a structurally complex class of oligomers and polymers. Previous studies have shown that certain PACs can reinforce dentin selectively, improve the dentin-resin bond strength, and are suitable agents for primary caries prevention.[12,14-17]

With the median life span of resin composite restorations being eight years in adults,[18] multiple replacements are likely in the lifetime of a patient. Every time a replacement is made, more tooth structure is lost, and as a result, repeated failure and replacement of restorations can lead to premature tooth loss. The purpose of the present study was to assess the protective effects of bioactive agents against secondary caries development around enamel and dentin margins of class V resin composite restorations, using an *in vitro* bacterial caries model. The null hypothesis was that bioactive primers do not affect secondary caries development around enamel and dentin margins compared with control groups.

METHODS AND MATERIALS

Materials

The chemical agents used in the study were as follows: 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid powder (HEPES, Sigma-Aldrich, St. Louis, MO, USA); 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC/NHS; Thermo Scientific Pierce, Rockford, IL, USA), *N*-hydroxysuccinimide (Thermo Scientific Pierce), and chlorhexidine digluconate (CHX) stock solution (Alfa Aesar, Ward Hill, MA, USA).

Grape seed extract was obtained from Polyphenolics Inc. (Madera, CA, USA) and consisted of PACs with a degree of polymerization (DP) ranging from oligomers (2 to 7) up to polymers (8 to >20). Using a previously published method,[19] the polymeric PACs were selectively depleted from the crude extract to yield an enriched oligomeric mixture (e-GSE). The refined e-GSE material was composed of phenolic acids and phenolic monomers (PACs) that are commonly known as catechins and the oligomeric PACs. Gravimetrically, approximately 70% of the e-GSE fraction consisted of flavan-3-ol monomers; the remaining 30% was oligomeric PACs (OPACs). Among the OPACs in e-GSE, approximately 50% are dimers, and the other half are mid- to high-order OPACs. Both classes of compounds are jointly referred to as PACs in the following.

Restorative Procedures and Specimen Preparation

Bovine incisors were placed in 0.1% thymol solution for four weeks. Teeth were cleaned to remove debris, periodontal ligament, and cementum of the root surfaces, and then they were visually inspected and excluded if enamel defects and white spots were detected with a magnifying dental loupe. Teeth were sectioned 4 mm above and 4 mm below the cemento-

enamel junction (CEJ) at the mid-mesial and mid-distal surfaces using a diamond wafering blade (Buehler- Series 15LC Diamond, Buehler, Lake Bluff, IL, USA). Teeth were further sectioned into two halves to obtain mesial and distal sections to a final rectangular dimension of 8 mm width × 8 mm length × 1.5-2 mm thickness. Class V preparations of 3 mm width × 3 mm length × 1 mm depth were cut at the CEJ using a flat-end carbide bur (#558, Brasseler USA Dental, Savannah, GA, USA) in a high-speed handpiece with air/water coolant. Enamel and root dentin margins were prepared at 90° to the tooth surface, and burs were changed every five preparations.

Cavity preparations were randomly assigned to four groups (n=15). For the control group (HEPES primer), cavity walls were etched with 32% phosphoric acid (Scotchbond, 3M ESPE, St Paul, MN, USA) for 15 seconds and rinsed with distilled water for 30 seconds. Preparations were blotted dry with an absorbent tissue (KimWipe, Kimberly-Clark Corporation, Irving, TX, USA) and primed with 20 mM HEPES buffer for one minute, rinsed for 30 seconds, and blotted dry with an absorbent tissue, and a drop of Adper Single Bond Plus (3M ESPE) was actively applied on the preparation surfaces. The adhesive layer was air dried to remove excess solvent and light cured for 20 seconds (Optilux 501 light unit at 830 mW/cm^2, Kerr, Orange, CA, USA). Preparations were filled with Filtek Supreme Plus Universal composite material (3M ESPE) in two vertical increments and light cured for 40 seconds each. Immediately after the final curing, restorations were polished with coarse-, medium-, and fine-grit aluminum-oxide abrasive discs (Sof-Lex, 3M/ESPE) in a slow speed handpiece.

The experimental groups followed the same restorative sequence, except for the following protocols for each primer: e-GSE primer, priming solution containing 15 w/v% e-GSE was applied for one minute and rinsed with distilled water for 30 seconds, modified from Castellan and others[20]; EDC/NHS primer, priming solution containing 0.3 M EDC/0.12 M NHS was applied for one minute[21] and rinsed for one minute; and CHX primer, priming solution containing 2% chlorhexidine was applied for 30 seconds and blotted dry.[22]

Artificially Induced Secondary Caries

Cosmetic nail varnish was applied 1 mm away from the margins of the restorations and air dried for 40 minutes. Specimens were disinfected in 70% ethanol for 20 minutes,[23] rinsed with sterile phosphate buffered saline (PBS) twice, and stored in sterile PBS at 4°C overnight. *Streptococcus mutans* UA159 was aerobically cultured on Brain Heart Infusion (BHI, Difco Laboratories, Detroit, MI, USA) agar, and a colony was inoculated into BHI broth and incubated for 18-20 hours at 37°C. Then, cells were washed twice with PBS and suspended in fresh medium supplemented with 1% sucrose (BHIS) and standardized to 1×10^8 cells/mL spectrophotometrically (absorbance of 0.20 at 550 nm; Spectronic 601, Milton Roy, Ivyland, PA, USA). Specimens were inoculated with *S. mutans* suspension in BHIS for 4 hours at 37°C, after which the media were replaced with BHI without sucrose for the next 20 hours (modified protocol from Fontana and others).[24] Wells were gently rinsed with PBS buffer twice following each media change. At the end of a four-day challenge, specimens were removed from the wells and rinsed in running water thoroughly. Specimens were sectioned along the axis of the tooth, through the restorations. Sections were embedded in epoxy resin overnight and polished with #320, #400, #600, #800, and #1200 grit silicon carbide abrasive papers (Buehler) under running water.

Fluorescence Microscopy Analysis

Specimens were hydrated with distilled water for one hour and stained overnight with 0.1 mM rhodamine B solution (pH 7.2), following the protocol described by Fontana and others.[25] After elapsed time, specimens were rinsed in running water for one minute and blotted dry with absorbent paper. Specimens were examined under a fluorescence microscope (DMI 6000 B, Leica, Buffalo Grove, IL, USA) with a connected digital camera (Hamamatsu, Skokie, IL, USA) and LAS AF software (Leica). Images of light differential interference contrast (DIC) microscopy, as well as red fluorescence at 529 nm, were captured. The same microscope settings were used for all images. Images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Positions of restoration margins were identified in DIC images and transferred to fluorescence images. Lesion depth (LD) was measured 125 μm away from the restoration margin as the depth of rhodamine stained from the surface. Secondary caries was measured as total fluorescence (TF; Figure 1),[25] where a fluorescent area was marked and TF was measured as area multiplied by mean fluorescence. For dentin, TF was measured within 250, 100, 50, or 25 μm from the restoration. For enamel, TF was measured within 250 or 25 μm from the restoration.

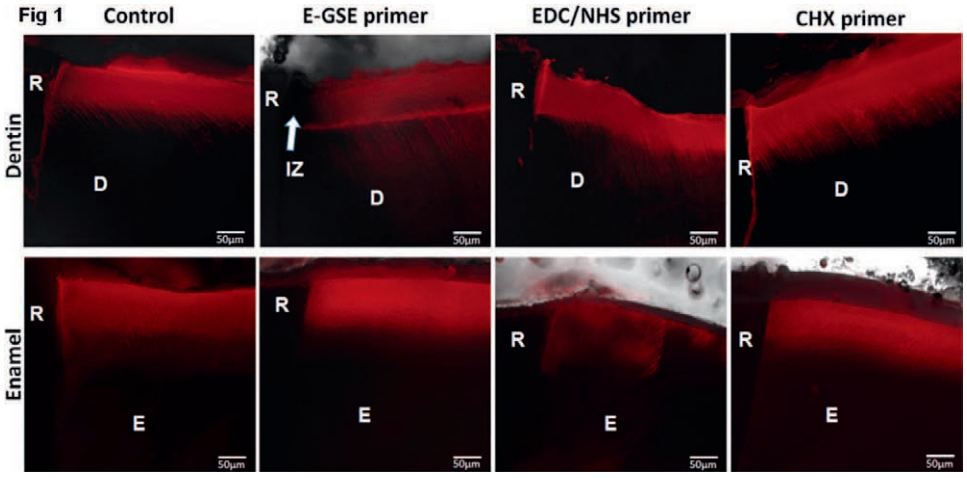

Figure 1. *Representative images of rhodamine-B–infiltrated dentin and enamel depicting demineralization around class V restorations restored with bioactive primers. R, resin; D, dentin; E, enamel; IZ, inhibition zone.*

Data were analyzed for the effect of treatment on LD and TF by one-way analysis of variance and Bonferroni post hoc test at α=0.05.

RESULTS

Margins in Dentin

LDs were similar across the treatment groups (Figure 1); there were no statistically significant mean differences in LDs (Table 1; $p>0.05$). Most interestingly, an inhibition zone (IZ) was noted in the e-GSE group, where rhodamine staining was scarce next to the tooth-resin interface (Figure 1). Such IZ was not observed in the control or any other treatment group. When examined up to 250 μm adjacent to the restoration, there was no statistically significant difference in total fluorescence among all treatment groups ($p>0.05$). When the area was limited to 100 or 50 μm adjacent to the restoration, a statistically significant difference was observed between the e-GSE and CHX groups ($p<0.05$). When the area was limited to 25 μm adjacent to the restoration, total fluorescence for the e-GSE group was significantly lower than all the other groups (Table 1). The e-GSE group had the least amount of demineralization, especially near the restoration margin, which was consistent with the presence of an inhibition zone (Figure 1). The e-GSE primer showed a protective effect immediately adjacent to the dentin-resin interface.

Margins in Enamel

LDs in enamel were similar across the experimental groups as shown in Table 1 ($p>0.05$) and Figure 1. Regardless of the areas examined, 250 or 25 μm adjacent to the restoration, there were no statistically significant mean differences in TF among all groups (Table 1). Enamel demineralization did not differ across the treatment groups ($p>0.05$).

DISCUSSION

An *S. mutans*–induced caries model was used for artificial caries development, which was clinically more relevant than a pH-cycling model. Carious lesions in dentin were more aggressive closer to the restoration (distance 100 vs 300 μm). The current findings confirm that dentin-resin interface seems to be more susceptible to caries progression around the restoration margin than the enamel-resin interface,

Table 1: *Secondary Caries Lesions Depth and Total Fluorescence Calculated by Area and Distances From the Dentin and Enamel Margins*[a]

Cavity primers	Margins in dentin					Margins in enamel		
	Lesion depth (μm)	Total fluorescence ($\times10^5$), mean (SD)				Lesion depth (μm)	Total fluorescence ($\times10^5$), mean (SD)	
		250 μm	100 μm	50 μm	25 μm		250 μm	25 μm
Control	72.5 (9.8)	8.06 a (5.51)	3.37 ab (1.98)	1.82 ab (1.04)	1.04 b (0.57)	80.5 (29.7)	6.20 (6.93)	4.16 (3.71)
e-GSE	74.8 (15.0)	8.23 a (5.57)	2.29 a (1.63)	0.79 a (0.70)	0.27 a (0.30)	67.8 (18.7)	4.06 (3.50)	2.20 (1.37)
EDC/NHS	71.2 (16.4)	10.75 a (6.78)	4.45 ab (3.14)	2.29 ab (2.05)	1.24 b (1.14)	71.7 (22.6)	2.42 (3.90)	2.40 (2.79)
CHX	74.4 (13.1)	12.29 a (6.27)	4.86 b (2.85)	2.69 b (1.81)	1.37 b (0.83)	83.4 (49.3)	5.55 (6.43)	3.21 (3.12)

[a] *Different letters indicate statistically significant differences in each column ($p<0.05$).*

which may be associated to less effective bonding to dentin compared with enamel. Resin composite's surface roughness and hydrophobicity[26,27] may have favored adhesion of oral streptococci and contributed to more demineralization closer to the dentin margins. The integrity of the tooth-resin interface influences the progression of caries around the restoration margin, with microleakage providing an additional portal for bacterial attack.[28,29]

One of the most interesting results of this study was that the e-GSE primer inhibited secondary caries development immediately adjacent to the dentin-resin interface. The e-GSE protective effect against secondary caries development was clearly represented by the presence of an inhibition zone, as well as the lowest total fluorescence relative to all other groups, measured within 25 μm of the restoration (Table 1). Three possible mechanisms of actions can be considered for e-GSE to inhibit secondary caries in dentin: (1) tissue stabilization, (2) a tighter interfacial seal, and (3) antimicrobial activity. As dentin is relatively porous, the e-GSE PACs were able to diffuse further than a few micrometers of the hybrid layer and protect dentin beyond the interface. Collagen cross-linking has been suggested to stabilize dentin by providing a scaffold for mineralization and a barrier for acid diffusion and mineral loss.[14,16] The observed fluorescence patterns suggest that surface caries lesions progressed to the peripheries until limited by PAC-treated dentin near the interface.

A tighter resin seal is achievable at the dentin-resin interface when the collagen mesh structure is intact and capable of forming a hybrid layer.[30] The e-GSE primer showed these properties, inducing collagen cross-linking and maintaining the collagen mesh structure before application of the bonding agent. A tighter interfacial seal is considered to be more resistant to bacterial leakage and acid diffusion; previous studies reported a positive correlation between the interface gap size and secondary caries.[31-33] Studies have suggested that 250- to 400-μm gaps contribute to the development of secondary caries and do not consider an open margin as an indication for replacement of a restoration.[4] However, gaps of 50 μm and less were previously shown to be colonized by *S. mutans* biofilm and subsequently caries being formed specifically at the interface.[28,29] An additional study will be required to assess the effect of e-GSE on marginal integrity without a caries challenge.

PACs are known for general antimicrobial properties. Specific to cariogenic bacteria, PACs inhibit surface-adsorbed glucosyltransferases and acid production by *S. mutans*,[34] as well as decrease the growth of *S. mutans* and biofilm formation.[35] Catechins such as epigallocatechin gallate suppress *gtf* genes associated with *S. mutans* biofilm formation.[36] The antimicrobial activity of PACs against cariogenic bacteria likely contributes to the inhibition of dentin demineralization by e-GSE, as the dentin tissue might function as a reservoir for PACs bound to the collagen backbone.

All other agents evaluated in this study had no significant effect on inhibiting secondary caries formation around resin composite restorations. Although EDC has collagen cross-linking activity,[37] it did not have the same effect as e-GSE. EDC's cross-linking ability is known to be less potent than other chemical agents,[13,38] and no effect was observed on interfacial nanoleakage compared with a control.[38] Although EDC was shown to inhibit bacterial membrane ATPases[39] and sugar uptake in oral streptococcal bacteria,[40] EDC does not take part in newly induced cross-linkage and is quickly hydrolyzed in solution, thus limiting a significant antimicrobial protection at the adhesive interface.

CHX was not found to inhibit secondary caries in any of the outcomes. A possible explanation is that CHX does not exhibit a permanent binding mechanism to the dentin structure and that the residual amount of CHX at the interface was too low for exerting bactericidal activity as it is only bacteriostatic at low concentrations.[41] Furthermore, a few studies have suggested weakening of the tooth-resin bond by chlorhexidine.[42,43]

As with any *in vitro* study, cautions remain when extrapolating results to the actual oral environment. The bacterial caries model in this study involved only a single species of cariogenic bacteria (*S. mutans*). However, the present design provides significant and promising findings. Inevitably, the hybrid layer is subjected to degradation and fatigue over time.[30,44] Aging of specimens was not simulated in the present work, but will be pursued in future studies.

CONCLUSIONS

Bacterial-induced secondary caries develops in enamel and dentin regardless of the composition of the primer solution. Lesions were more aggressive closer to the dentin-resin adhesive margins. An enriched fraction of grape seed extract, e-GSE, significantly inhibited secondary caries development within 25 μm of the restoration margin in dentin.

None of the other treatments inhibited secondary caries development around resin composite restorations. The results suggest that incorporation of e-GSE in the restorative procedure of resin composites may reduce secondary caries development immediately around highly susceptible root dentin margins.

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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 Illinois at Chicago.

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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Comparison of Internal Adaptation in Class II Bulk-fill Composite Restorations Using Micro-CT

SH Han • SH Park

Clinical Relevance

A flowable bulk-fill composite can demonstrate inferior internal adaptation compared with that of a packable bulk-fill composite. Internal adaptation may be worse at the gingival floor of the proximal box and at the pulpal floor of the cavity than it is at the buccal and lingual walls of the proximal box.

SUMMARY

Purpose: This study compared the internal adaptation of bulk-fill composite restorations in class II cavities and explored the relationship between internal adaptation and polymerization shrinkage or stress.

Methods and Materials: Standardized mesio-occluso-distal cavities were prepared in 40 extracted human third molars and randomly divided into five groups (n=8). After having been applied by total-etch XP bond (Dentsply Caulk, Milford, DE, USA) and light curing, the teeth were restored with the following resin composites: group 1, Filtek Z350 (3M ESPE, St. Paul, MN, USA); group 2, SDR (Dentsply Caulk, Milford, DE, USA) + Z350; group 3, Venus Bulk Fill (Heraeus Kulzer, Dormagen, Germany) + Z350; group 4, Tetric N-Ceram Bulk Fill (Ivoclar Vivadent, Schaan, Liechtenstein); and group 5, SonicFill (Kerr, West Collins, Orange, CA, USA). After thermo-mechanical load cycling, cross-sectional microcomputerized tomography (micro-CT) images were taken. Internal adaptation was measured as imperfect margin percentage (IM%), which was the percentage of defective margin length relative to whole margin length. On the micro-CT images, IM% was measured at five interfaces. Linear polymerization shrinkage (LS) and polymerization shrinkage stress (PS) were measured on each composite with a custom linometer and universal testing machine. To explore the correlation of IM% and LS or PS, the Pearson correlation test was used.

Results: The IM% of the gingival and pulpal cavity floors were inferior to those of the cavity walls. The IM% values of the groups were found to be as follows: group 5 $\leq$ groups 1 and 4 $\leq$ group 2 $\leq$ group 3. The correlation analysis showed that the p value was 0.006 between LS and IM% and 0.003 between PS and IM%, indicating significant correlations ($p<0.05$).

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Conclusion: Flowable bulk-fill composites had a higher IM% and polymerization shrinkage stress than did packable bulk-fill and hybrid composites. In class II composite restoration, the gingival floor of the proximal box and pulpal floor of the cavity had higher IM% than did the buccal and lingual walls of the proximal box. LS and PS, which were measured under compliance-allowed conditions, were significantly related to internal adaptation.

INTRODUCTION

To fill a large cavity with composite resin, the composite can be placed by incremental layering or bulk filling. To reduce polymerization shrinkage stress, the incremental technique has been recommended.[1] The incremental technique can be used to reduce C-factor and allow a certain amount of flow to decrease the shrinkage stress.[2,3] Bulk filling has been reported to yield significantly higher cuspal deflection than incremental filling when the composites were sufficiently cured.[4,5] The incremental filling technique showed higher bond strength to the floor in a large cavity than did the bulk filling technique.[2,6,7] A previous study compared microleakage of a composite restoration performed with the two techniques.[8] Gingival microleakage from bulk filling was significantly greater than that from incremental filling. Another reason for recommending an incremental technique is the compromised ability of light to penetrate the resin composite. Even when the composite surface is adequately cured, the base of composites thicker than 2 mm may not be completely polymerized, especially with micro- or nano-hybrid composites of a darker shade.[9-11]

In the last few years, new resin composites have been introduced for bulk filling. Bulk filling technology allows placement of up to 4- or 5-mm thick resin composite in a single fill, without compromising polymerization efficiency.[12,13] Bulk-fill resins can be divided into two categories: flowable and packable. The first bulk-fill materials on the market, such as SDR (Dentsply Caulk, Milford, DE, USA), Venus Bulk Fill (Heraeus Kulzer, Dormagen, Germany), x-tra base (Voco GmbH, Cuxhaven, Germany), and Filtek Bulk Fill (3M ESPE, St. Paul, MN, USA), needed a capping layer of micro- or nano-hybrid composite resin.[14] Later bulk-fill resins, like SonicFill (Kerr, West Collins, Orange, CA, USA), Tetric EvoCeram Bulk Fill (Ivoclar Vivadent, Schaan, Liechtenstein), and x-tra fil (Voco), can be placed without a capping layer. SonicFill can be placed with the help of a sonic-activated handpiece.

Many studies have evaluated microleakage from bulk-fill composite restorations.[15-17] Moorthy and others evaluated cuspal deflection and cervical microleakage on the margins of class II cavities that had been incrementally filled with composites or flowable bulk-fill resins.[15] They found that cuspal deflection was lower with flowable bulk-fill resin, whereas cervical microleakage did not differ between the composites. Roggendorf and others evaluated the marginal integrity of bonded composite resin fillings on mesio-occluso-distal (MOD) cavities with and without a flowable bulk-fill 4-mm base.[16] They inspected the marginal gap using scanning electron microscopy (SEM) and found no negative influence on marginal quality when a 4-mm layer of bulk-fill SDR was used. Campos and others investigated the marginal adaptation of bulk-fill composite restorations on class II mesio-occlusal cavities and concluded that bulk-fill materials do not allow better marginal adaptation than a standard composite resin applied by simple layering technique.[17]

Internal adaptation can be defined as how well a restoration adapts internally to the dental substrate. Internal refers to the location of the interface between a resin and tooth material inside. Internal adaptation may be related to hypersensitivity to cold or pain on mastication.[18] To evaluate internal adaptation or microleakage, dye and tracer penetration has been used.[19] However, the specimens need to be sectioned in these techniques, and this procedure might lead to false interfacial leakage.[19] The resin-dentin interface can also be examined by SEM after sectioning.[20] This method is very technique sensitive and has limitations with regard to quantitative assessment. Therefore, as a nondestructive method to evaluate internal adaptation, microcomputerized tomography (micro-CT) was introduced.[21] The two-dimensional (2D) information from micro-CT can be reconstructed into three dimensions to evaluate internal adaptation.[22,23] Because micro-CT is nondestructive, a specimen can be evaluated repeatedly before and after aging.[24] Due to the penetrating capacity of x-rays, micro-CT can be used to examine the internal aspects of a restoration irrespective of a specimen's shape or dimensions.

Because flowable resin was introduced as a restorative material, the advantages of flowable resin as a base material compared with composite restoration have been questioned. The rheology of flowable resins could allow better adaptation to cavity walls.[25] Flowable resin layers have been suggested to act as a stress-absorbing layer.[26]

Kwon and Park reported that the use of flowable composites of low elastic modulus as the base material could reduce marginal defects in overlying composite restorations.[27] Braga and others reported that using a flowable resin composite as a restorative material is not likely to reduce the effects of polymerization stress.[28] Therefore, it has been questioned if placing a base of flowable bulk-fill resin could improve internal adaptation at the resin-tooth interface.

The purpose of this study was to compare the internal adaptation of class II resin restorations filled with different bulk-fill resins. Internal adaptation was compared to analyze differences depending on location or material. We also investigated whether internal adaptation was correlated with polymerization shrinkage or stress.

The null hypotheses of this study were (1) the internal adaptation of class II composite restorations did not differ at different resin-tooth interface locations; (2) the internal adaptation of class II composite restorations did not differ with different bulk-fill composite materials; and (3) the internal adaptation was not correlated with the linear polymerization shrinkage or polymerization stress of the resin composite.

METHODS AND MATERIALS

Specimen Preparation

Forty caries-free, sound, lower third molars that had been extracted within the three previous months were collected and stored in 0.5% chloramine solution. The sizes of the specimen teeth were controlled so that the differences in bucco-lingual and mesio-distal length were less than 1 mm. The teeth were randomly divided into five groups, and standard MOD cavities were prepared with diamond burs (959 KR 018; Komet Dental, Lemgo, Germany). The cavity was 4.5 mm deep in the central fossa area, and the bucco-lingual isthmus was 3.5 mm wide. The proximal box of the cavity was prepared on the mesial side of the tooth. The cervical margin of the mesial proximal box was 1 mm below the cemento-enamel junction (CEJ), and the cervical margin of the distal proximal surface was 1 mm above the CEJ. Cavity dimensions were controlled by meticulously preparing the teeth with a magnifying glass and digital caliper. After the teeth were prepared, all roots were resected at 2 mm below the CEJ. Detailed dimensions of the cavity are depicted in Figures 1 and 2.

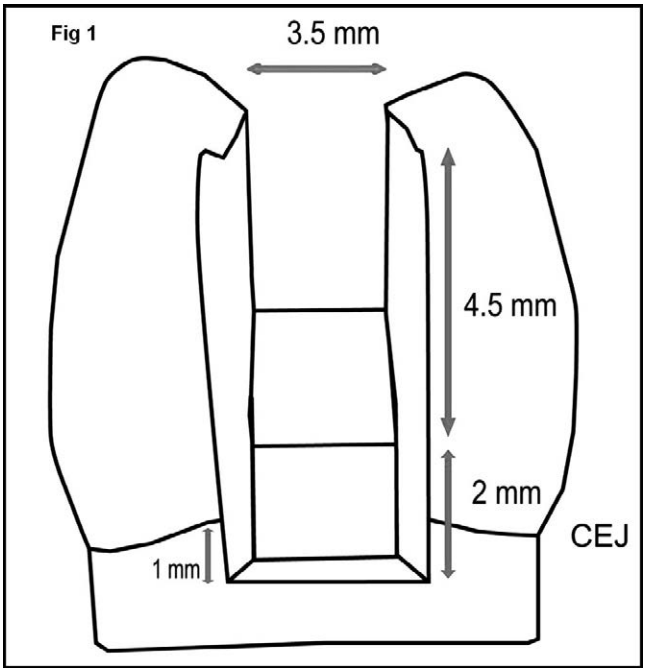

Figure 1. *Tooth preparation from the mesial view.*

Base and Composite Filling

The materials used in this study are shown in Table 1. All specimens were cleaned with distilled water after they were prepared. Enamel margins were etched with 34% phosphoric acid (Dentsply Caulk) for 15 seconds, irrigated with distilled water for 15 seconds, and then air-dried for 15 seconds according to the manufacturer's instructions. After XP bond (Dentsply Caulk) was applied to each cavity, the resin was light-cured 1 mm away from the occlusal, mesial, and distal sides for 20 seconds each with an LED-type light source (Bluephase, 800 mW/cm^2, Ivoclar Vivadent). Resin composite was placed after the adhesive treatment. Five different resin composites were used.

Group 1 (Z3, Control Group)—The control group was restored with a nano-hybrid composite, and the filling technique used was incremental layering. After the ivory retainer and matrix were applied to the tooth specimen, Filtek Z350 composite resin (A3, 3M ESPE) was applied. The first 2-mm increment was placed in the mesial proximal box and light-cured for 20 seconds from the occlusal side. The second and third 2-mm increments were placed in the mesial proximal box and the pulpal floor, respectively, and then light-cured for 20 seconds each from the occlusal side. The fourth and fifth increments were placed in the mesial and distal half of the remaining cavity, respectively, and were light-

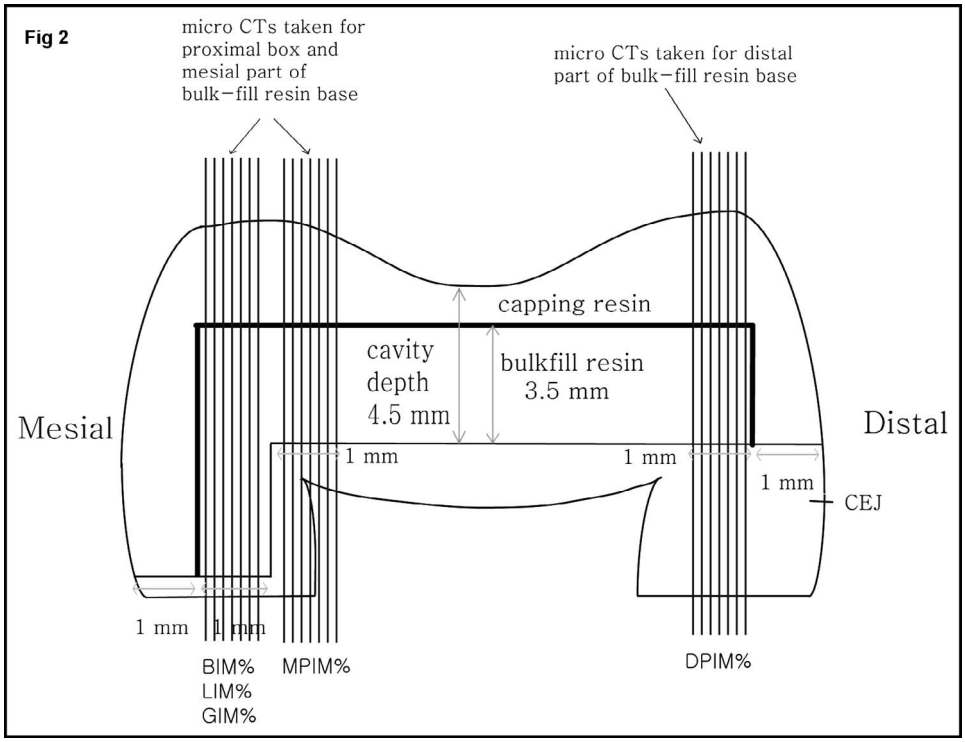

Figure 2. *Bulk-fill resin placement and locations where micro-CT images were taken. BIM%, buccal wall imperfect margin %; LIM%, lingual wall imperfect margin %; GIM%, gingival floor imperfect margin %; MPIM%, mesial pulpal floor imperfect margin %; DPIM%, distal pulpal floor imperfect margin %; CEJ, cemento-enamel junction.*

cured for 20 seconds each from the occlusal side. After removing the retainer and matrix, additional light-curing was applied from the buccal and lingual sides of the mesial and distal cavities (20 seconds × 2 for mesial side and 20 seconds × 2 for distal side).

Group 2 (SD, Flowable Bulk-fill Resin) and Group 3 (VB, Flowable Bulk-fill Resin)—After an ivory retainer and matrix band were placed on the specimen, flowable bulk-fill resin was used to fill the base portion of each cavity. For group 2, SDR (Dentsply Caulk) bulk-fill resin was placed on the mesial proximal box and light-cured. Resin was then placed on the pulpal floor at a thickness of 3.5 mm and light-cured (Figure 2). The base materials and cavity walls were refined with a fine diamond bur, hand instruments, and digital calipers to control the base thickness. After the refining process, the base surface was cleaned with cotton and dry air. Z350 composite was added to the remaining cavity: first on the mesial proximal, second on the distal proximal, third on the mesial cusp portion, and fourth on the distal cusp portion. Light-curing was performed for 20 seconds after each application. After removing the retainer and matrix, additional light-curing was performed from the buccal and lingual sides of the mesial and distal cavities (20 seconds × 2 for mesial side and 20 seconds × 2 for distal side). For group 3, Venus Bulk Fill (Heraeus Kulzer) was placed as the base material. The base dimension, refining, and light-curing processes were the same as for group 2. A capping layer of Z350 composite was added to the

Table 1: *Compositions of Resin Composites in This Study*[a]

Code	Product	Manufacturer	Base resin	Filler (wt/vol.%)
Z3	Filtek Z350	3M ESPE, St Paul, MN, USA	Bis-GMA/EMA, UDMA	78.5/59.5%
SD	SDR	Dentsply Caulk, Milford, DE, USA	Modified urethane dimethacrylate	68/44%
			EBPADMA/ TEGDMA	
VB	Venus Bulk Fill	Heraeus Kulzer, Dormagen, Germany	UDMA, EBPDMA	65/38%
TB	Tetric N-Ceram Bulk Fill	Ivoclar Vivadent, Schaan, Liechtenstein	Bis-GMA, UDMA	78/55% (including prepolymer)
			Dimethacrylate co-monomers	
SF	SonicFill	Kerr, West Collins, Orange, CA, USA	Bis-GMA, TEGDMA, EBPDMA	83.5/68%

[a] *Composition and filler percentages are from the manufacturers.*
Abbreviations: BIS-EMA, ethoxylated bisphenol A dimethacrylate; BIS-GMA, bisphenol A glycidyl methacrylate; EBPDMA, ethoxylated bisphenol A dimethacrylate; TEGDMA, triethyleneglycol dimethacrylate; UDMA, urethane dimethacrylate.

remaining cavity. Light-curing was performed for 20 seconds after each application. After removing the retainer and matrix, additional light-curing was performed from the buccal and lingual sides of the mesial and distal cavities (20 seconds × 2 for mesial side and 20 seconds × 2 for distal side).

Group 4 (TB, Packable Bulk-fill Resin) and Group 5 (SF, Packable Bulk-fill Resin)—After an ivory retainer and matrix band were placed on the specimen, packable bulk-fill resin was used to fill each cavity in groups 4 and 5. For group 4, Tetric N-Ceram Bulk Fill (Ivoclar Vivadent) was placed with hand instruments at the same dimensions as in groups 2 and 3, followed by light-curing for 20 seconds. Another layer of Tetric N-Ceram Bulk Fill composite was added to the remaining cavity and light-cured in the same way as with groups 2 and 3. For group 5, SonicFill (Kerr) was applied as filler with a sonically activated handpiece following the manufacturer's instructions. After SonicFill resin was filled to the full cavity depth, light-curing was performed for 20 seconds. After removing the retainer and matrix, additional light-curing was applied from the buccal and lingual sides of the mesial and distal cavities (20 seconds × 2 for mesial side and 20 seconds × 2 for distal side).

Thermo-mechanical Load Cycling

After storing in water for 24 hours, the specimens were mechanically loaded with a chewing simulator CS-4.8 (SD Mechatronik, Feldkirchen-Westerham, Germany). They were then simultaneously thermocycled under thermodynamic conditions (5-55°C, with a dwell-time of 60 seconds and a transfer time of 24 seconds) and a mechanical load of 5 kgf (49 N) for 600,000 cycles. A conical-shaped opposing plunger made of nickel-chromium was initially positioned at the center of the restoration. A 5-kgf load was applied from the top surface and pressed into the center of the tooth. In the Zurich wear testing study, a 5-kgf (49 N) load applied for 1,200,000 cycles with thermo-cycling of 5-55°C was equivalent to five years of service *in vivo*.[29] The rod moved 6 mm vertically and 0.3 mm horizontally. The rising speed was 55 mm/s, whereas the descending speed was 30 mm/s. After thermo-mechanical loading, the samples were stored in distilled water at room temperature.

Silver Nitrate Infiltration

Silver nitrate was used to fill microgaps.[24] Precipitated silver nitrate functioned as a contrasting medium on x-ray images.[30] The specimens were soaked in 17% EDTA for five minutes to remove the smear layer. Then they were rinsed with distilled water. The teeth were completely immersed in a 25% ammoniacal silver nitrate solution and placed under 3.75-kPa pressure, upside down, for 3 days. This step facilitated silver nitrate infiltration from the pulp chamber into the cavity floor. The specimens were then rinsed thoroughly with distilled water and kept in water at room temperature.

Micro-CT Imaging

Thirty cross-sectional micro-CT images were taken of each specimen. Ten micro-CT images of the proximal box were taken, extending from the mesial end of the bulk filled base to the axial wall of the proximal box at 90-μm intervals (Figure 2). Ten micro-CT images were taken at the mesial end of the bulk filled cavity floor. The last 10 micro-CT images were taken from the distal end of the bulk filled base toward the center of the cavity at the same interval (Figure 2). A high-resolution micro-CT (Model 1076, Skyscan, Aartselaar, Belgium) was used to obtain images. The imaging settings were as follows: acceleration voltage, 100 kV; beam current, 100 μA; Al filter, 0.5 mm; resolution, 18 μm; and rotation, 360° in 0.5° steps. Each tooth was mounted on a specially designed jig for uniform imaging. The 2D images were analyzed with image analysis software (ImageJ ver. 1.46, National Institutes of Health, Bethesda, MD, USA).

Evaluation of Internal Adaptation

To evaluate internal adaptation, the silver spots that were present between the tooth and restoration were measured on the micro-CT images. All the images were captured from the mesial point of view. Figure 3 shows an image at the mesial proximal box, and Figure 4 shows an image taken at a nonproximal box portion of the cavity. To evaluate internal adaptation, a previously reported method was applied.[30]

The imperfect margin percentage (IM%) was calculated by dividing total length where silver nitrate had penetrated the microgap by the entire length of the wall or floor. The local IM% was calculated for each image. On the proximal box images, the buccal wall imperfect margin percentage (BIM%), lingual wall imperfect margin percentage (LIM%), and gingival floor imperfect margin percentage (GIM%) were measured (Figures 2 and 3). Mesial pulpal floor imperfect margin percentage (MPIM%) was measured from micro-CT images captured from the mesial end of pulpal floor 1 mm toward the center of the cavity (Figures 2 and 4). Distal pulpal floor imperfect margin percentage

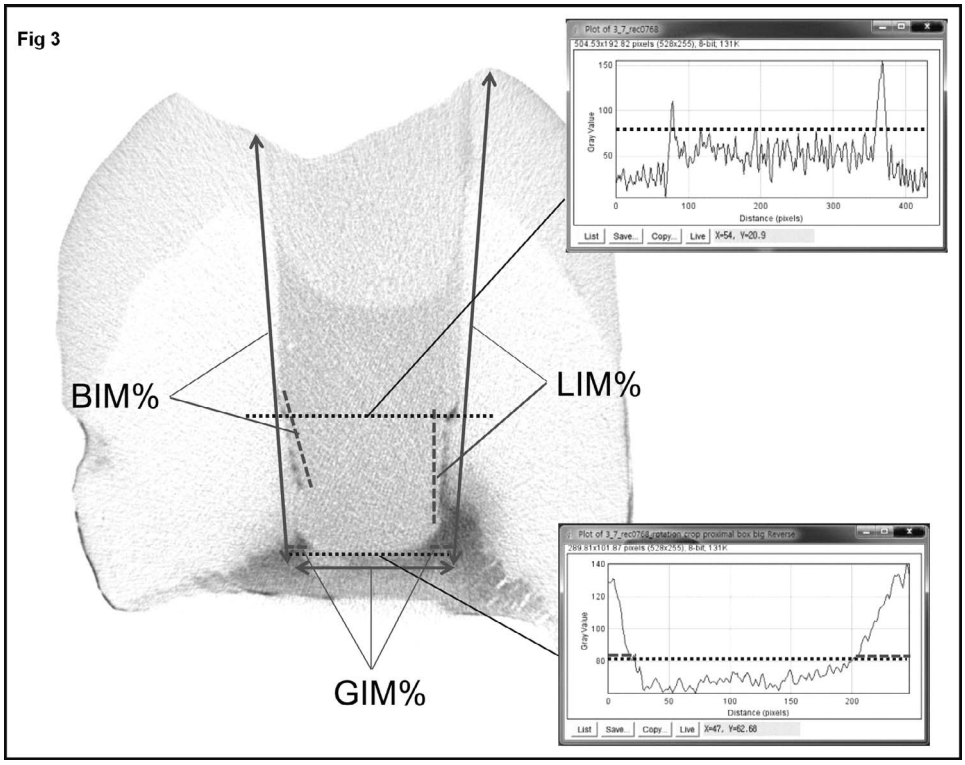

Figure 3. *Measurement of the imperfect margin for BIM%, LIM%, and GIM% on micro-CT. BIM%, buccal wall imperfect margin %; LIM%, lingual wall imperfect margin %; GIM%, gingival floor imperfect margin %. The plot profile shows the intensity of the pixels along the horizontal axis. The plot profile function in ImageJ shows a gray density of the selected line or area. Dentin exhibits a gray density of 20-40, bulk-fill resin has a gray density of 40-70, and the silver nitrate infiltrating gaps shows a gray density of >80 (upper right inset). Intensity >80 was regarded as a critical value for determining the imperfect area (lower right inset).*

(DPIM%) was measured on micro-CT images captured from the distal end of the bulk-filled base.

To verify any correlation between polymerization shrinkage and internal adaptation, the total imperfect margin percentage (TIM%) was calculated, defined as the total imperfect margins on the buccal wall, lingual wall, gingival floor, mesial pulpal floor, and distal pulpal floor divided by the sum of all internal margins in a specimen.

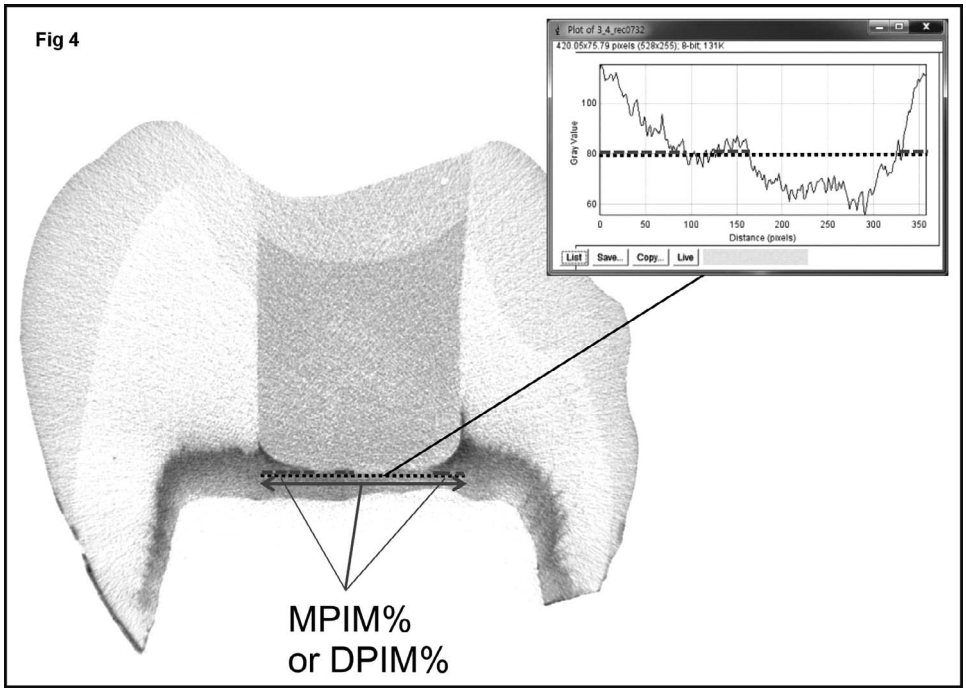

Figure 4. *Measurement of the imperfect margin for MPIM% and DPIM% on the pulpal floor of the cavity. MPIM%, mesial pulpal floor imperfect margin %; DPIM%, distal pulpal floor imperfect margin %. The plot profile shows the intensity of the pixels along the horizontal axis. Intensity >80 was regarded as a critical value for determining the imperfect area (upper right inset).*

Table 2: *Physical Properties of the Resin Composites Used in This Study*[a]

Group	Flexural modulus (GPa)[b]	Linear polymerization shrinkage (%)	Polymerization shrinkage stress (MPa)
1(Z3)	6.32 (0.65)^{D}	1.36 (0.10)^{A}	2.19 (0.12)^{A,B}
2(SD)	3.04 (0.63)^{B}	1.78 (0.12)^{B}	3.02 (0.17)^{C}
3(VB)	1.10 (0.15)^{A}	2.27 (0.11)^{C}	3.46 (0.18)^{D}
4(TB)	5.33 (0.43)^{C}	1.20 (0.09)^{A}	2.03 (0.12)^{A,B}
5(SF)	6.03 (0.45)^{C,D}	1.21 (0.12)^{A}	1.86 (0.15)^{A}

[a] *Polymerization stress was measured under a compliance-allowed condition. Numbers in parentheses are SDs. Identical letters within each column indicate no statistically significant difference among groups,* $p > 0.05$ *(compared vertically).*
[b] *Flexural modulus measurements were adopted from the literature.*

The definitions of the terms are as follows: BIM%, buccal wall imperfect margin percentage = (length of the buccal wall of the proximal box that was penetrated by silver nitrate/height of the buccal wall of the proximal box) × 100; LIM%, lingual wall imperfect margin percentage = (length of the lingual wall of the proximal box that was penetrated by silver nitrate/height of lingual wall of the proximal box) × 100; GIM%, gingival floor imperfect margin percentage = (length of the gingival floor of the proximal box that was penetrated by silver nitrate/width of gingival floor of proximal box) × 100; MPIM%, mesial pulpal floor imperfect margin percentage = (length of the mesial pulpal floor of the occlusal cavity that was penetrated by silver nitrate/width of pulpal floor of the cavity) × 100; DPIM%, distal pulpal floor imperfect margin percentage = (length of the distal pulpal floor of the occlusal cavity that was penetrated by silver nitrate/width of pulpal floor of the cavity) × 100; TIM%, total imperfect margin percentage = (sum of the imperfect margin measurements on the buccal wall, lingual wall, gingival floor, mesial pulpal floor, distal pulpal floor/total length of all internal margins) × 100.

Measuring Polymerization Linear Shrinkage of Resin Composite (LS)

Polymerization shrinkage strain was measured with a custom-made Linometer (R&B Inc, Daejon, Korea).[24] Glycerin gel was applied to a metallic disc and a portion of a glass slide to prevent adhesion to the resin. Resin composites were measured in a custom Teflon mold to ensure that the same amount of composite resin (0.07 g, 29.45 mm^2; 5 mm diameter x 1.5 mm high disc) was used for each linometer sample. The composite was then transferred to the thin metallic discs, covered with a glass slide, and positioned under constant pressure from a screw. An LED-type light-curing unit (Bluephase, Ivoclar Vivadent) with a power density of 800 mW/cm^2 was placed 1 mm above the glass slide, and the material was light-cured for 30 seconds. As the resin composite under the glass slide was cured, the aluminum disk moved upward. The disc displacement due to linear shrinkage of the resin composite was measured with an eddy current sensor every 0.5 seconds for 180 seconds. This measurement was repeated five times for each material, and the average was calculated (Table 2).

Measurement of Polymerization Shrinkage Stress Under a Compliance-allowed Condition (PS)

The polymerization shrinkage stress of the resin composite was measured with a custom-made device and software (R&B Inc). The instrument was driven by a motor and was devised to move a load bar up and down. The polymerization shrinkage stress applied to the bar was measured by a load cell (Model UM-K100, capacity 100 kgf, Dacell, Chungcheongbuk-do, South Korea) connected to the bar. The composite was placed between an acrylic disc, which was screwed to the load bar, and a transparent cylinder. Light to polymerize the resin composite was projected from beneath the transparent cylinder. The entire process was controlled by software by R&B Inc.

The surface of the acrylic disk was roughened with sandpaper before it was screwed to the load bar. The surface was coated with adhesive resin (Bond, Clearfil SE Bond, Kuraray Noritake Dental, Okayama, Japan) and light-cured for 20 seconds. To measure polymerization stress, a Teflon mold was used to measure a consistent amount of resin composite (0.03 g, 7.07 mm^2; 3-mm-diameter × 1-mm-high disc). The composite was placed on the acrylic disc, and the load bar screwed to the disk was moved so that the composite specimen formed a disk with a 1-mm thickness and a 3-mm diameter. Before measurements, the setup was switched to compliance-allowed mode in which the feedback system was not activated. The compliance of the system was 0.3 μm/N. The stress between the tension rod and the

Table 3: *Internal Adaptation Depending on Cavity Location (Result of One-Way ANOVA)*

Interface location	N	Subset[a]	
		1	2
Lingual wall (LIM%)	40	26.2175	
Buccal wall (BIM%)	40	26.6197	
Distal pulpal floor (DPIM%)	40		38.8276
Gingival floor (GIM%)	40		40.5827
Mesial pulpal floor (MPIM%)	40		41.6917

[a] *Subset 1 differed significantly from subset 2 (one-way ANOVA, p<0.05). In both subsets 1 and 2, there was no statistical difference between or among locations (p>0.05).*

Table 4: *Internal Adaptation of Groups (TIM%) (Results of One-Way ANOVA)*

Group	N	Subset[a]		
		1	2	3
5 (SF)	40	30.1221		
1 (Z3)	40	32.8954	32.8954	
4 (TB)	40	32.9378	32.9378	
2 (SD)	40		37.0317	37.0317
3 (VB)	40			40.9522

[a] *Subsets 1, 2, and 3 differed significantly (one-way ANOVA, p<0.05). In each subset, there was no statistical difference between or among groups (p>0.05).*

resin composite was set to zero before light-curing. Then the resin composite was light-cured with a light-curing unit (800 mW/cm^2, Bluephase, Ivoclar Vivadent) through the transparent disc for 20 seconds. Along with the load-cell signal, the displacement was continuously recorded by the computer every 0.1 seconds for 180 seconds. This measurement was repeated five times for each material, and the average was calculated (Table 2).

Statistical Analysis

Statistical analysis was conducted with PASW Statistics 18 software (SPSS for Windows, SPSS Inc, Chicago, IL, USA). To compare internal adaptation, two-way analysis of variance (ANOVA) was applied. To compare internal adaptations among different groups or those of different locations, one-way ANOVA with Scheffe analysis was used. Pearson correlation test was used to analyze the relationships between polymerization parameters and internal adaptation (IM%).

RESULTS

Table 2 shows physical properties of the resin composites used in the study. The linear polymerization shrinkages were 4(TB), 5(SF), and 1(Z3) < 2(SD) < 3(VB). The polymerization shrinkage stresses were 5(SF) and 4(TB) ≤ 1(Z3) < 2(SD) < 3(VB).

Two-way ANOVA results showed no interaction between filling materials and locations ($p>0.05$). One-way ANOVA with Scheffe analysis indicated that GIM% (gingival floor), MPIM%, and DPIM% (pulpal floor) were significantly higher than BIM% and LIM% ($p<0.05$; Table 3). There was no significant differences among GIM%, MPIM%, and DPIM% ($p>0.05$). The IM% values of the different groups were as follows: group 5 ≤ groups 1 and 4 ≤ group 2 ≤ group 3 (Table 4). The detailed results in Table 5 were compared by one-way ANOVA, horizontally (among different filling materials) and vertically (among different locations). Pearson correlation analysis showed that p was 0.006 between LS and IM% and 0.003 between PS and IM%, indicating that internal adaptation was significantly correlated with both polymerization shrinkage and stress ($p<0.05$).

DISCUSSION

GIM%, MPIM%, and DPIM% were significantly higher than BIM% and LIM%, and TIM% differed among groups. These findings indicate that the first and second null hypotheses were rejected. The third hypothesis was rejected due to the correlation between internal adaptation and polymerization shrinkage parameters.

GIM% was relatively higher than BIM% and LIM% in the proximal box. This difference might be due to the occluso-gingival height of the proximal box, which was longer than the bucco-lingual width of the box. The dimensions were approximately 6 mm for the occluso-gingival height and 3.5 mm for the bucco-lingual width. As the bulk resin polymerized, polymerization shrinkage could be greater in the occluso-gingival axis than in the bucco-lingual axis. This difference could lead to a higher IM% at the gingival floor interface. While measuring imperfect margins at the restoration interfaces, imperfections were often found around the axiofaciogingival or axiolinguogingival point-angles of the proximal box. This may be because these two point-angles are where more than two vectors of force are exerted: toward the occlusal surface and toward the buccal/lingual surface. Another explanation for the difference in IM% is that the buccal and lingual walls contained their own portions of the enamel margin. The enamel part of the cavity could bond more

Table 5: *Mean Percentage of Measured Imperfect Margin (IM%) of Each Cavity Wall or Floor*[a]

Location	Group 1	Group 2	Group 3	Group 4	Group 5
BIM%	27.8^{a,A,B} (4.24)	28.3^{a,B,C} (4.07)	29.9^{a,C} (4.52)	24.4^{a,A,B} (5.03)	23.4^{a,A} (4.24)
LIM%	25.2^{a,A,B} (5.94)	27.0^{a,B,C} (4.02)	29.7^{a,C} (3.48)	25.3^{a,A,B} (5.90)	23.9^{a,A} (3.6)
GIM%	37.7^{b,A,B} (4.98)	43.4^{b,B,C} (3.33)	48.5^{b,C} (3.28)	38.9^{b,A,B} (5.29)	34.5^{b,A} (3.51)
MPIM%	38.0^{b,A,B} (3.22)	45.1^{b,B,C} (3.11)	50.2^{b,C} (2.97)	40.1^{b,A,B} (2.85)	35.1^{b,A} (2.74)
DPIM%	36.5^{b,A,B} (3.77)	41.3^{b,B,C} (4.14)	46.4^{b,C} (5.06)	36.2^{b,A,B} (3.30)	33.7^{b,A} (2.73)
TIM%	32.9^{A,B} (6.07)	37.0^{B,C} (8.67)	40.9^{C} (10.3)	32.9^{A,B} (7.56)	30.1^{A} (5.93)

[a] *Numbers in parentheses are SDs. Lowercase superscripts represent differences in IM% among the locations where the imperfect margin was measured within each group at* $p<0.05$ *(compared vertically). Capital superscripts represent differences in IM% among the groups at each location at* $p<0.05$ *(compared horizontally). Identical letters represent no statistical difference at* $p>0.05$*. BIM%, buccal wall imperfect margin %; DPIM%, distal pulpal floor imperfect margin %; GIM%, gingival floor imperfect margin %; LIM%, lingual wall imperfect margin %; MPIM%, mesial pulpal floor imperfect margin %; TIM%, total imperfect margin %.*

strongly where little microleakage occurred along the enamel margin on the micro-CT images. After resin polymerization, the composite restoration might have produced a strong bond at the occlusal margin and a weak bond at the cervical margin. Intact enamel margins could have decreased the IM% of the buccal and lingual walls. Finally, dentinal tubules on a deep cavity floor have different characteristics from those of outer superficial dentin. The tubule diameters near the pulpo-dentinal junction (PDJ) are larger, the distance between tubule centers is half the distance between tubule centers at the dentino-enamel junction (DEJ), and peritubular dentin is either thinner or absent.[31] At the PDJ, the volume of the fluid-filled tubule lumens approaches 80%.[32] Therefore, the dentin at this area is more permeable and wetter than dentin at the DEJ.[33] These conditions are poor for dentin bonding, especially with an etch-and-rinse system like the XP-bond used in this experiment. Therefore, the gingival and pulpal floors might be less favorable to dentin bonding than are the occlusal portions of the buccal and lingual walls.

Polymerization shrinkage and stress are thought to be major factors for the difference in IM% among groups. The different resin composites seemed to have their own polymerization shrinkage characteristics. Groups 2 and 3 (SD and VB), which were flowable bulk-fill composite resins, had high linear polymerization shrinkage and stress. They also had higher IM%, indicating that internal adaptation and polymerization were significantly correlated. The composites with low filler content (wt/vol%) had higher IM% (Tables 1 and 4). Filler content appears to be one of the most important material properties affecting IM%.

The magnitude of shrinkage stress is influenced by numerous factors like shrinkage volume, elastic modulus, flow of the resin, filler contents, resin matrix formulation, adherence of the resin to the wall, compliance of the cavity wall, and C-factor.[34-36] The interplay between these factors can determine the shrinkage stress of the restoration. According to the linear elastic model, the change in shrinkage stress is proportional to the product of increase in volumetric shrinkage and change in elastic modulus.[3] In the present study, information on the flexural modulus was adopted from a previous study from our laboratory (Table 2). The flexural modulus increased as Group VB $<$ SD $<$ TB $\leq$ SF $\leq$ Z3, whereas the linear polymerization shrinkage increased in a different order: groups TB, SF, and Z3 $<$ SD $<$ VB. This difference could indicate that polymerization shrinkage stress depends much more on polymerization shrinkage than on elastic modulus. Compliance in the measuring system might offset the influence of the elastic modulus, and its effect on stress might be limited. Therefore, the polymerization stress of these flowable bulk-fill resins did not decrease due to low elastic modulus but demonstrated high shrinkage stress. Another report demonstrated that polymerization stress had a strong direct correlation with shrinkage and an inverse correlation with elastic modulus.[37]

There are two types of measurement systems for polymerization shrinkage stress measurement: a zero compliance (rigid) setup and a compliance-allowed (nonrigid) setup.[38,39] Because the nonrigid setup does not have a feedback system, composite resin can shrink relatively freely under the compliance-allowed condition. With the nonrigid setup, shrinkage stress can dissipate through the components of the system.[39] In the present study, polymerization stress was measured under compliance-allowed condition because some compliance was found in the MOD cavity due to the cuspal deflection.[5]

Light-curing of bulk-fill composites can be affected by several variables of the light source: distance, angle, output, and footprint. Depth of cure was

linearly correlated with distance.[40] Versluis and others used finite element analysis to show that polymerization shrinkage vectors differed depending on the incidence angle of the light source.[41] Peutzfeldt and Asmussen found that not only energy density (the product of output and exposure time), but also power density (output) had a significant influence on resin properties.[42] Sufficient light energy is needed to create sufficient double bonds for the composite to link to the adhesive layer.[42,43] Low irradiance generates a small number of free radicals, which results in longer polymeric chains with low cross-linking.[44] In this experiment, to minimize incomplete curing of the resin composite, additional light curing was performed after the matrix was removed.

In this study, the packable bulk-fill resins, TB and SF, had better internal adaptation than did the flowable bulk-fill resins. The TB manufacturer insisted that TB includes an initiator called Ivocerin that increases polymerization. They claim that TB also contains low-elastic modulus fillers that relieve shrinkage stress. SF uses sonic energy to place the composite. SF has a special modifier that can be activated by sonic waves. Once activated by sonic energy, SF viscosity decreases to obtain rheological properties for adaptation. When the polymerization shrinkage is similar, the material with the lower viscosity has better marginal adaptation.[45,46] In the SF group, however, small bubbles were found inside the resin restoration on micro-CT images. These bubbles could be due to the vibration of the resin composite by sonic energy. Although they might relieve stress, they could increase IM% if they were present at the interface between the composite and tooth.

The purpose of this paper was to compare the internal adaptation of bulk-fill composite restorations in class II cavities. Internal adaptation (IM%) was determined by a critical threshold of the gray value on micro-CT images. Setting a threshold level is somewhat subjective, so measurements of the imperfections do not represent absolute leakage. However, this method makes possible relative comparison. Bonding between the base and capping layer could have been affected by the refining process. To avoid removing the oxygen inhibition layer of the base material, the use of finishing burs was minimized. The capping layer was light-cured right after the refining process, which meant that the composites could continue to polymerize.[46] Even though the oxygen inhibition layer was reduced, the capping layer and bulk-fill base composites could have reacted because free radicals were present for polymerization.[47] To evaluate internal adaptation of the bulk-fill composite restoration, improved methods and further studies are needed.

CONCLUSION

Flowable bulk-fill composites had a higher IM% and polymerization shrinkage stress than did packable bulk-fill and hybrid composites. In class II composite restorations, the gingival floor of the proximal box and pulpal floor of the cavity had higher IM% than did the buccal and lingual walls of the proximal box. Polymerization shrinkage and stress, which were measured under compliance-allowed conditions, were significantly related to internal adaptation.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Yonsei University Dental Hospital Institutional Review Board. The approval code for this study is number 12-0149.

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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Adhesive Cementation Promotes Higher Fatigue Resistance to Zirconia Crowns

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Clinical Relevance

In this study, the adhesively cemented zirconia crowns needed more load and cycles to failure. Therefore, adhesive cementation should be the strategy of choice for luting zirconia crowns.

SUMMARY

Objective: The aim of this study was to investigate the influence of the cementation strategy on the fatigue resistance of zirconia crowns. The null hypothesis was that the cementation strategy would not affect the fatigue resistance of the crowns.

Methods and Materials: Seventy-five simplified molar tooth crown preparations were machined in glass fiber–filled epoxy resin. Zirconia crowns were designed (thickness=0.7 mm), milled by computer-aided design/computer-aided manufacturing, and sintered, as recommended. Crowns were cemented onto the resin preparations using five cementation strategies (n=15): ZP, luting with zinc phosphate cement; PN, luting with Panavia F resin cement; AL, air particle abrasion with alumina particles (125 μm) as the crown inner surface pretreatment + Panavia F; CJ, tribochemical silica coating as crown inner surface pretreatment + Panavia F; and GL, application of a thin layer of porcelain glaze followed by etching with hydrofluoric acid and silanization as crown inner surface

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pretreatment + Panavia F. Resin cement was activated for 30 seconds for each surface. Specimens were tested until fracture in a stepwise stress fatigue test (10,000 cycles in each step, 600 to 1400 N, frequency of 1.4 Hz). The mode of failure was analyzed by stereomicroscopy and scanning electron microscopy. Data were analyzed by Kaplan-Meier and Mantel-Cox (log rank) tests and a pairwise comparison ($p<0.05$) and by Weibull analysis.

Results: The CJ group had the highest load mean value for failure (1200 N), followed by the PN (1026 N), AL (1026 N), and GL (1013 N) groups, while the ZP group had the lowest mean value (706 N). Adhesively cemented groups (CJ, AL, PN, and GL) needed a higher number of cycles for failure than the group ZP did. The groups' Weibull moduli (CJ=5.9; AL=4.4; GL=3.9; PN=3.7; ZP=2.1) were different, considering the number of cycles for failure data. The predominant mode of failure was a fracture that initiated in the cement/zirconia layer. Finite element analysis showed the different stress distribution for the two models.

Conclusion: Adhesive cementation of zirconia crowns improves fatigue resistance.

INTRODUCTION

Nowadays, among dental ceramics, zirconia has the greatest fracture toughness[1] and flexural strength.[2] In addition, this material has the "transformation toughening" mechanism, in which its grains turn from a tetragonal to monoclinic phase, with volumetric expansion, to prevent crack propagation.[2] Therefore, this material can be used for crown infrastructures and fixed partial dentures (FPDs). Most recently, this material has also been used for full-contour indirect restorations of posterior regions, so that chipping failures, which are the most common failure type in veneered crowns,[3] do not occur.[4]

Zirconia is a crystalline material that cannot be etched by hydrofluoric acid (at low concentrations) as can the glass-based ceramics.[5] Thus, the adhesion between the tooth and the zirconia crown is a critical point. Hence, several surface treatments have been proposed to improve the resin bonding to zirconia: air particle abrasion with alumina or alumina coated with silica particles (tribochemical silica coating),[6,7] application of an etchable glaze layer,[8,9] use of 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based primers,[10,11] plasma deposition of silica films,[12] and many others. In addition, the use of MDP-based resin cements has been indicated.[13] However, a recent literature review stated that resin bonding to zirconia is no longer a drawback of this material, since the cementation strategies—with surface mechanical treatments and chemical approaches—that have been applied to improve this bonding interface seem to be reliable.[14]

In addition to retention, luting with resin cement leads to less microleakage[15] and, in glass-ceramic restorations, to incomplete fractures.[16] Despite this, nonadhesive cementation strategies are still recommended for more retentive zirconia preparations, such as crowns and FPDs.[17] The hypothesis that the high fracture strength of the zirconia could support the entire restoration, no matter which cement is used, guides this recommendation.[17] Indeed, surface damage is more common in bilayer restorations.[18] However, the complete fracture of zirconia crowns has been reported.[19,20] Moreover, as the restoration is a complex mechanical system including the ceramic bilayer crown, the cement, and the tooth, all of the components and the interaction between them are important. The question is, how do Y-TZP crowns behave in terms of fatigue resistance when different cementation approaches are used?

Therefore, the aim of this study was to investigate the influence of the cementation strategy on the fatigue resistance of zirconia crowns. The null hypothesis was that the cementation strategy would not affect the fatigue resistance of the crowns.

METHODS AND MATERIALS

Prosthetic Preparation and Zirconia Crown Production

A simplified posterior full-crown preparation (6-mm high, large chamfer finishing line, 12° of convergence of the walls) was designed, and 75 replicas were machined in glass fiber–filled epoxy resin. This epoxy resin has an elastic behavior similar to human dentin[21] (National Electrical Manufacturers Association [NEMA] grade G10, Accurate Plastics, New York, NY, USA).

A preparation model was first scanned with a laboratory scanner (inEos Blue, Sirona Dental, Bensheim, Germany). The framework was virtually designed and machined from zirconia blocks (Vita InCeram 2000 YZ, Vita Zahnfabrik, Bad Säckingen, Germany) using a CAD/CAM system (Cerec MC XL, Sirona Dental, Bensheim, Germany) with 80 μm of cement space. After the sintering process (Zyrcomat

Table 1: *Loads and Number of Cycles for Failure for the Experimental Groups*[a]

Group	Load (N)				Number of Cycles			
	Mean	SE	95% Confidence Interval		Mean	SE	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
PN	1026[B]	47	934	1119	26,474[a]	2275	22,014	30,934
ZP	706[C]	33	641	771	9376[b]	1240	6945	11,808
GL	1013[B]	41	932	1094	24,319[a]	1870	20,653	27,985
AL	1026[B]	38	951	1101	25,788[a]	1746	22,364	29,212
CJ	1200[A]	33	1133	1266	32,532[a]	1745	29,110	35,953

[a] *The same uppercase letters denote no significant statistical difference. The same lowercase letters denote no significant statistical difference.*

T, Vita Zahnfabrik, Bad Säckingen, Germany), the crowns achieved the final thickness of 0.5 mm for the circumferential and 0.7 for the occlusal wall.

Luting Procedures

The crowns were cleaned with ethanol before the respective surface treatments and allocated into five groups, according to the cementation strategy:

- Group ZP: no zirconia surface treatment + zinc phosphate cement.
- Group PN: no zirconia surface treatment + resin cement.
- Group AL: air particle abrasion with alumina particles (125 μm) + resin cement. The crowns were air particle abraded (Rocatector delta, 3M ESPE AG, Seefeld, Germany) with alumina particles (Alublast 125 μm, Elephant Dental B.V., the Netherlands) with 3 bar of pressure during 15 seconds and with 15 mm of distance.
- Group CJ: air particle abrasion with alumina coated with silica particles (30 μm) + silane + resin cement. The crowns were air particle abraded as in the AL group, with the alumina coated by silica particles (CojetSand, 3M ESPE).
- Group GL: application of a glaze layer + etching with hydrofluoric acid + silane + resin cement. A thin layer of glaze ceramic (Vita Akzent, Vita Zahnfabrik, Bad Sackinger, Germany) was applied on the crowns' intaglio surface with a brush and then cured according to the manufacturer's recommendation. This glaze layer was etched with hydrofluoric acid at 9% for 1 minute and silanized.

For the ZP group, the preparations were ultrasonically cleaned in distilled water for 5 minutes before the cementation process. For the other groups, the surface preparations were treated with hydrofluoric acid at 9% for 1 minute, rinsed with distilled water, ultrasonically cleaned in distilled water for 5 minutes, and air dried. A silane layer (Clearfil Porcelain Bond Activator + Clearfil SE Bond Primer, Kuraray Medical, Tokyo, Japan) was applied on the preparation with a microbrush, followed by a gentle air stream. The adhesive system (ED primer, Kuraray Medical, Tokyo, Japan) was applied, followed by a gentle air stream, after 60 seconds.

For the ZP group, the zinc phosphate cement was mixed and applied according to the manufacturer's instructions. For the other groups, a dual-activated resin cement was mixed for 20 seconds and placed in the internal surface of each crown. These crowns were placed on the preparations, and a constant load of 50 N was applied during photo activation (Astralis 10, Ivoclar Vivadent AG, Schaan, Liechtenstein) of each surface for 30 seconds. The cemented crowns were then stored in distilled water for 1-7 days at 37°C.

Stepwise Stress Testing

The cemented crowns were tested until failure occurrence in a stepwise stress fatigue test. In each step of 10,000 cycles, a load of 600 to 1400 N (200 N of increment) was applied, with a frequency of 1.4 Hz, in an aqueous environment (Fatigue Tester, ACTA, Amsterdam, the Netherlands). The load was applied by means of a stainless steel piston ball of 40 mm in diameter.

Fracture Analysis

After fracture occurrence, the specimens were analyzed by stereomicroscopy (Olympus, Shinjuku, Tokyo, Japan) at a magnification of up to 100×. The specimens with the most significant failures were ultrasonically cleaned in isopropyl alcohol for 10 minutes, gold sputtered, and subjected to scanning electron microscopy (XL 20, FEI Company, GG Eindhoven, the Netherlands).

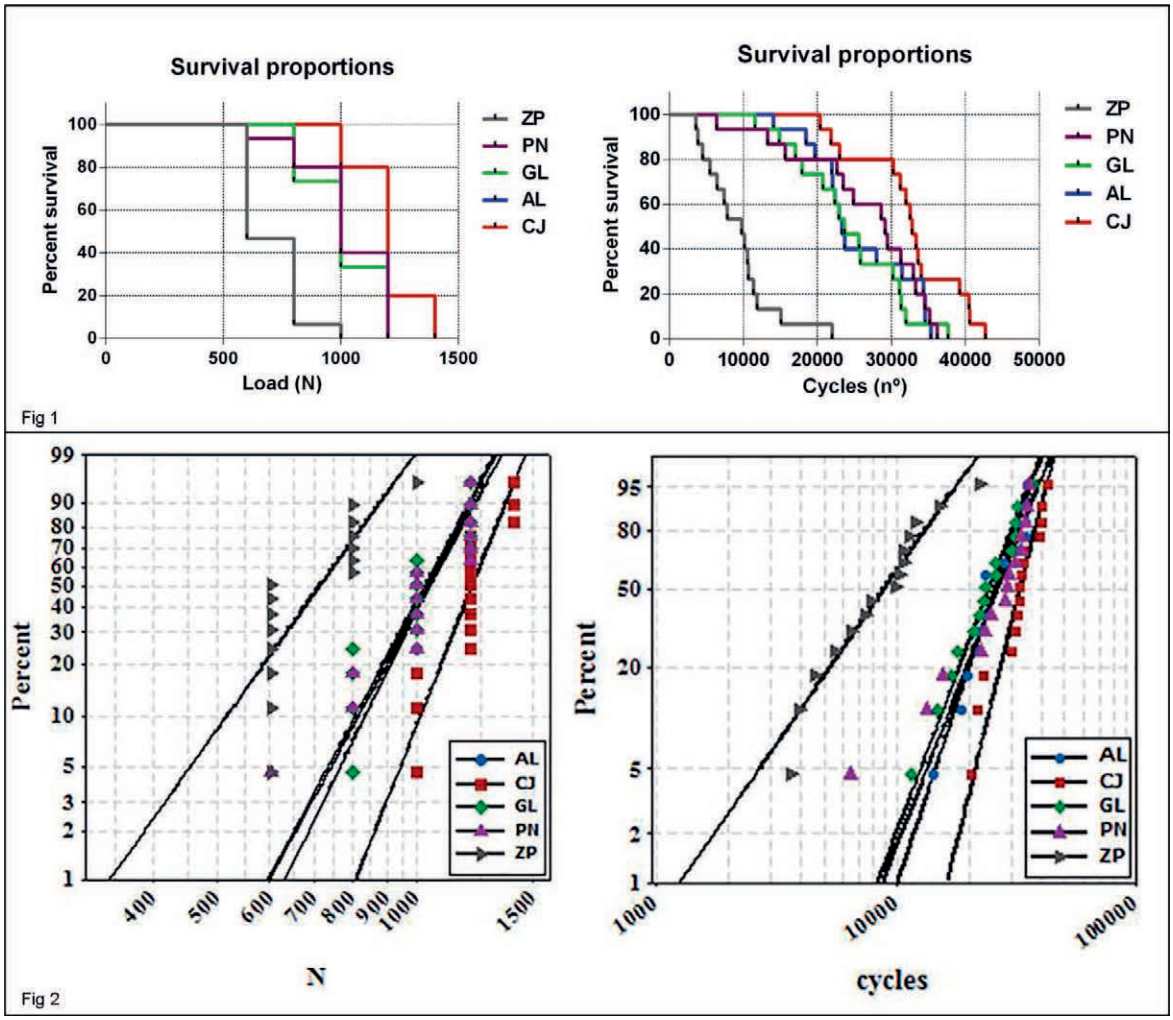

Figure 1. *Survival curves according to the steps of load (A, B) and number of cycles (C, D) in which each crown failed.*
Figure 2. *Weibull analysis according to the steps of load (A) and number of cycles (B) in which each crown failed.*

Finite Element Analysis

The finite element analysis (FEA) of the abutment with crown specimens was performed to evaluate the failure load values. Two models were made; in the first model, the bonding between the cement layer and the crown was supposedly strong enough to resist the shear stress in the cement layer–crown interface (model 1). In the second model, the surface in the interface between the preparation and the crown was modeled for contact surface purposes with a friction coefficient of 0.45 (model 2). Since the models are symmetric in two directions, a quarter FEA model was prepared to facilitate the boundary conditions using symmetry, with the nodes in the centric planes allowing sliding in the surface only. The FEA model was created using FEMAP software (FEMAP 10.1.1, Siemens PLM software, Plano, TX, USA), while the analysis was carried out with NX Nastran software (NX Nastran; Siemens PLM Software). The models consisted of 13,952 parabolic tetrahedron solid elements. For both models, calculations were made with the PN and ZP cement layers. The mechanical properties of the used materials were found in the literature: the Young's moduli (GPa) and the Poisson's ratios for zirconia, resin cement, zinc phosphate cement, and G10 were, respectively, 209.3 and 0.32,[22] 7 and 0.35,[23] 13.7 and 0.33,[24] and 14.9 and 0.31.[25] The nodes at the bottom of the abutment were fixed so that no movement was allowed in any direction. The crown was loaded on the nodes in the center of the occlusal surface, simulating the plastic deformation of the occlusal surface caused by the loading ball (radius of 20 mm). The calculations considered a load of 300 N.

Table 2: *Survival Rates (Probability That the Specimens Have to Exceed the Respective Load or Number of Cycles Without Failure) for the Experimental Groups*

Group	Load (N)					Number of Cycles[a]				
	600	800	1000	1200	1400	3500	12,000	22,000	32,000	40,560
PN	0.933 (0.064)	0.800 (0.103)	0.400 (0.126)	0.000 (0.000)	0.000 (0.000)	1	0.867 (0.088)	0.733 (0.114)	0.333 (0.122)	0.000 (0.000)
ZP	0.467 (0.129)	0.067 (0.064)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.933 (0.064)	0.133 (0.088)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
GL	1	0.733 (0.114)	0.333 (0.122)	0.000 (0.000)	0.000 (0.000)	1	0.933 (0.064)	0.060 (0.126)	0.067 (0.064)	0.000 (0.000)
AL	1	0.800 (0.103)	0.333 (0.122)	0.000 (0.000)	0.000 (0.000)	1	0.933 (0.064)	0.667 (0.122)	0.200 (0.103)	0.000 (0.000)
CJ	1	1	0.800 (0.103)	0.200 (0.103)	0.000 (0.000)	1	1	0.867 (0.088)	0.600 (0.126)	0.670 (0.640)

[a] *These numbers of cycles are approximated.*

Statistical Analysis

Data were analyzed with the application of Kaplan-Meier and Mantel-Cox (log rank) tests and a pairwise comparison ($p<0.05$; SPSS version 21, IBM, Chicago, IL, USA). Data were also examined using a Weibull analysis with two software packages (Minitab 17, State College, PA, USA, and Weibull++ 9, Reliasoft, Tucson, AZ, USA). For the Minitab 17 software, the Weibull parameters (shape and scale) were calculated in the maximum likelihood estimation method and the correlation coefficients were calculated in the least squares estimation method.

RESULTS

For the fracture load, a difference among the cementation strategies was detected (Mantel-Cox log-rank test, $X^2=56.50$, $p=0.000<0.05$; Table 1). The CJ group presented higher fracture load values, followed by the other adhesive strategies. Apart from that, for the number of cycles to fracture, a difference was also detected (Mantel-Cox log-rank test, $X^2=92.34$, $p=0.000<0.05$; Table 1). In this case, all of the adhesively cemented groups (CJ, AL, PN, and GL) needed a greater number of cycles for fracture occurrence than the ZP group, nonadhesively cemented.

Figure 1 shows the survival curves according to the steps of load and number of cycles until failure. Table 2 summarizes the mean fracture loads and number of cycles until failure, calculated from the survival curves.

Figure 2 shows the Weibull curves for the experimental groups. The Weibull parameters are described in Table 3. For the load to failure values, there is no difference in the Weibull modulus values or shape among the groups ($p=0.031$). On the other hand, there is a statistical difference in Weibull modulus values among the groups according to the number of cycles to failure ($p=0.007$).

Contour plots of the groups with 95% of bilateral confidence interval are presented in Figure 3. The "β values were higher than 1 (PN=3.7; ZP=1.8; GL=3.9; AL=4.4; CJ=5.9), indicating that failures occurred by fatigue in all groups.

Table 3: *Weibull Parameters With Their 95% Confidence Intervals (Maximum Likelihood Estimation) and Correlation Coefficient Least Squares Estimation[a]*

Group	Load			Number of Cycles		
	Shape (CI)	Scale (CI)	R^2	Shape (CI)	Scale (CI)	R^2
PN	7.6^{A} (4.9-11.6)	1079 (1023-1176)	0.94	3.7^{ab} (2.4-5.9)	29,293 (25,514-33,631)	0.93
ZP	5.7^{A} (4.0-8.3)	760 (692-834)	0.80	2.1^{b} (1.4-3.1)	10,629 (8284-13,637)	0.97
GL	7.6^{A} (5.1-11.5)	1079 (1007-1157)	0.91	3.9^{ab} (2.6-5.8)	26,908 (23,477-30,839)	0.99
AL	8.4^{A} (5.6-12.6)	1088 (1021-1159)	0.91	4.4^{ab} (2.9-6.5)	28,337 (25,107-31,983)	0.96
CJ	10.3^{A} (7.0-15.2)	1257 (1193-1323)	0.89	5.9^{a} (3.9-8.8)	35,155 (32,135-38,458)	0.96

[a] *The same uppercase letters denote no significant statistical difference. The same lowercase letters denote no significant statistical difference.*

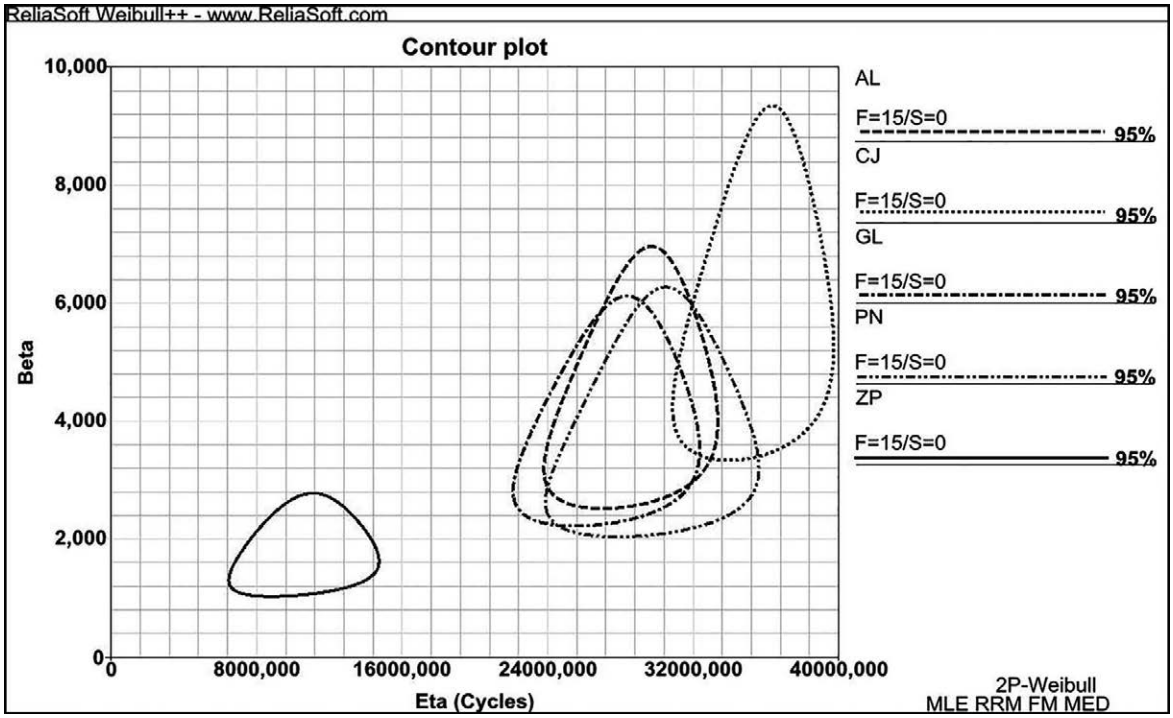

Figure 3. *Contour plots with 95% of bilateral interval confidence.*

The predominant mode of failure was a fracture that initiated in the cement/zirconia layer (Figure 4).

FEA and the stress values are shown in Figure 5 and Table 4, respectively.

DISCUSSION

The present study demonstrated that the cementation strategy affects fatigue resistance of zirconia-based crowns. Consequently, the null hypothesis was rejected, since the fatigue experiment showed that adhesively cemented crowns had higher survival rates than nonadhesively cemented ones. The FEA also confirmed these findings, as the stress distribution was different for bonded and nonbonded crown designs.

For porcelain-based crowns, it is known that the use of resin cements may increase the fracture resistance by means of blunting the defects of the ceramic restorations.[26,27] However, for alumina-, zirconia-, and lithium disilicate–based crowns, it is not clear if the luting adhesive enhances their mechanical properties, since some studies state there is no influence[17,28,29] while others show some influence.[30,31] A recent study[31] suggested that for veneered zirconia crowns, the cementation surface treatment (with sandblasting, glazing, or tribochemical silica coating) did not affect the fatigue resistance. However, the results of the cited study showed better performance for the groups cemented without previous surface treatment and with MDP-based resin cement. As this study[31] was carried out to produce veneering failures, such as chipping, it was necessary to develop a study in which the failure of the zirconia layer would be assessed. The present study was designed with this purpose and directed failures to the cementation region. In fact, the fractographic analysis showed that the fractures initiated on the interface between the cement and the zirconia, underneath the load application point (Figure 4).

The cementation strategies used in this study were in accordance with several bond strength studies,[14,32-34] in which different surface treatments—such as sandblasting of alumina or alumina coated with silica particles (tribochemical silica coating), and glaze layer application (ceramic coating)—and luting agents (zinc phosphate cement or resin cement with MDP) were used to promote the retention of zirconia-based restorations to the tooth substrate. Although the zinc phosphate cement does not produce chemical bonding to zirconia, it is still indicated for cementation of restorations with larger adherence areas available, such as crowns, because of their retention ability.[35,36] For this reason, this cement was used with the control group of this study. Although the application of a glaze layer in the intaglio surface before cementation of zirconia has been reported as an efficient treatment for bond

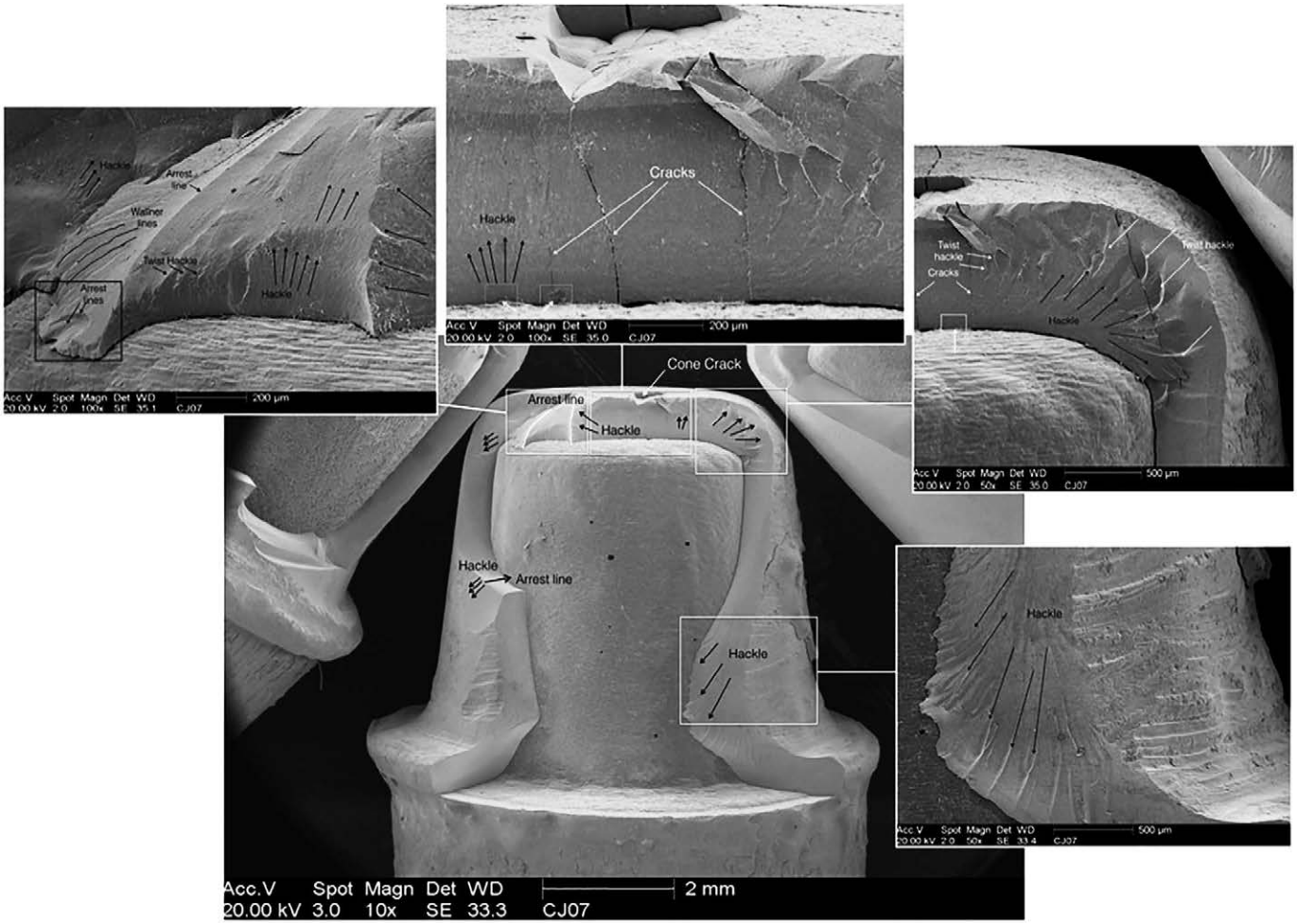

Figure 4. *Representative crown failure modes. The black arrows indicate failure direction. The white arrows indicate failure origins.*

strength improvement between zirconia and resin cement,[8,9] according to Yener and others,[37] this technique may lead to lower fracture strength results. In the present study, the GL group showed similar results compared with the adhesively cemented groups.

The adhesive cementation with sandblasting of alumina particles has been done for zirconia due to the promotion of a more retentive surface by the creation of a rougher topography.[6] Since some studies affirm this surface treatment could damage the zirconia's mechanical properties,[38] mainly with the use of larger alumina particles, in the present study, large alumina particles (125 μm) were used to simulate the worst possible scenario. However, even with this particle size, the AL group did not behave inferiorly when compared with the other groups. Probably, the combination of resin cement with MDP maintained the bond strength to zirconia, and consequently, the system behaved as a bonded crown. Similarly, the silicatization approach, which left some

Table 4: *The Maximum Tensile Stress (Solid Maximum Principal Stress) in the Zirconia and Maximum Tensile Stress and the Maximum Shear Stress (Solid Y Normal Stress) in the Cement Layer in the Circumferential Wall*

FEA Results	Model 1 (Bonded) PN	Model 2 (Nonbonded) ZP
Max tensile stress (MPa) (zirconia)	308	443
Max tensile stress (MPa) (cement)	6	85
Max shear stress (MPa) (cement)	7.5	56

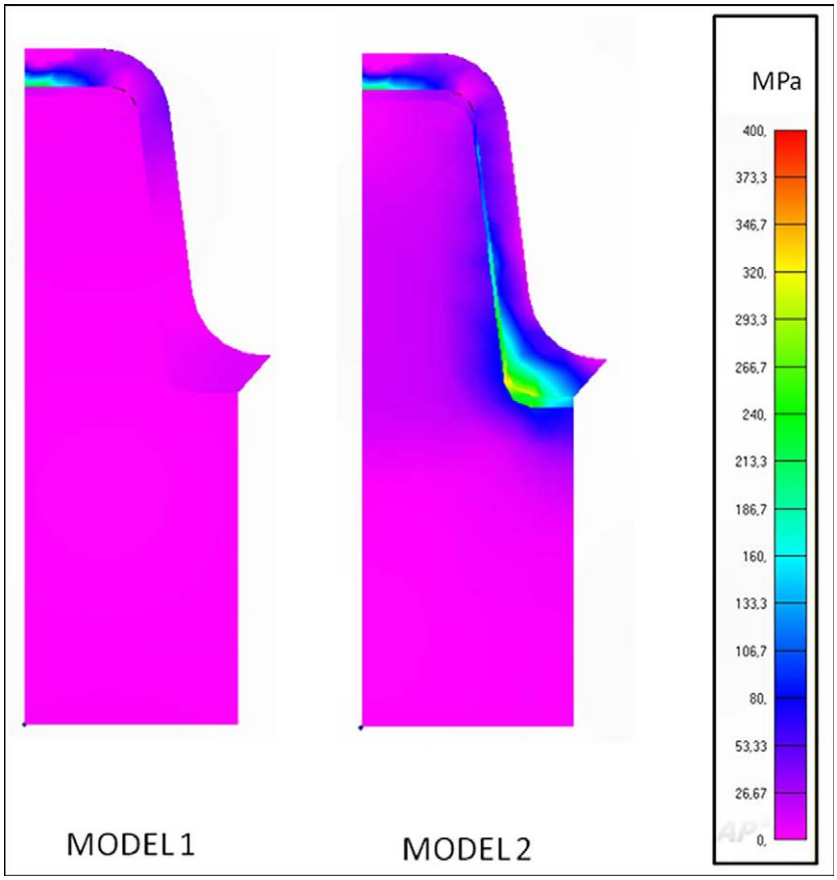

Figure 5. *The maximum principal stress in model 1 with the PN cement layer and in model 2 with the ZP cement layer.*

silica adhering to the zirconia surface by a tribochemical reaction, is the best treatment to improve the bond strength between zirconia ceramic and resin cement,[39,40] and the system acted as a bonded crown in the CJ group. However, the CJ specimens survived for more cycles in the higher load and presented the highest Weibull moduli. It is possible to state that this adhesive luting combination (tribochemical silica coating and resin cement with MDP) seems to be the more reliable cementation strategy.

Even though the origin of fracture of all crowns was in the bonding interface, the macroscopic failure mode was different among the groups. Most fragments of the groups adhesively cemented remained bonded to the resin abutment, while the fragments of the non–adhesively cemented group (ZP) were detached. This showed that the cementation strategies were simulated by FEA under closely realistic conditions, whereas the retention condition without chemical bonding (zinc phosphate cement) was applied as a nonbonded interface area with a friction coefficient of 0.45, resulting in an area with some friction, values of which may range between 0 (no friction) and 1 (without movement).

Considering that long periods of underwater storage may lead to lower bond strength, resin cement elastic moduli reduction, and changes in the stress distribution in the crowns/cement/tooth complex,[41] the short storage period is a limitation of the present study.

CONCLUSIONS

The adhesive cementation of zirconia crowns yields to a significantly higher fatigue resistance for zirconia crowns.

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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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Departments

Errata:

It was brought to our attention that the Reviewer Thank You list that was published in issue six of Volume 41 was incomplete. The Staff sincerely apologizes for the error. A corrected and complete list is being published with this issue, and replaces that found in 41-6. Thank you to all reviewers who so selflessly give of their time and talents to help us provide this content to the dental community.

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Reviewers

Operative Dentistry, Inc. would like to thank our conscientious team of Reviewers for their hard work, tenacity, and dedication in the furthering of operative dentistry around the word. These individuals dedicate innumerable hours in reading, re-reading, and critiquing manuscripts. Submitted articles, accepted for publication or not, all benefit from these reviewers who help authors present their hard work, as well as verifying that the work we publish is scientifically accurate, clinically relevant, and professionally uplifting. We cannot thank these individuals enough for their contributions, but we do want to publically recognize them.

Be it known here, and throughout the world, that the following named individuals have contributed real and invaluable service to the profession of Dentistry by volunteering their time and talents to the cause of peer-review for Operative Dentistry, Inc.

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Effect of Adhesive Restoration and Bleaching Technique on the Concentration of Hydrogen Peroxide In the Pulp Chamber

V Cavalli • BG Silva • SB Berger • G Abuna • FC Marson • CPM Tabchoury • M Giannini

Clinical Relevance: Hydrogen peroxide is able to diffuse into the pulp chamber even in the absence of enamel restorations. Silorane adhesive restorations allow a higher amount of hydrogen peroxide into the pulp chamber, compared to non-restored teeth.

doi: http://dx.doi.org/10.2341/16-079-L

Fifteen-year Clinical Follow-up of Restoration of Extensive Cervical Resorption in a Maxillary Central Incisor

EG Reston • RPR Bueno • LQ Closs • J Zettermann

Clinical Relevance: This multidisciplinary approach allowed us to maintain the tooth and bone height, contributing to the patient's facial esthetics and obviating the need for more complex and invasive procedures, along with the psychological aspect of maintaining the tooth in the dental arch.

doi: http://dx.doi.org/10.2341/15-131-S

Interfacial Characteristics and Bond Durability of Universal Adhesive to Various Substrates

A Tsujimoto • WW Barkmeier • T Takamizawa • TM Wilwerding • MA Latta • M Miyazaki

Clinical Relevance: Clinicians should follow the procedures associated with universal adhesives to modify the interfacial characteristics carefully and pay attention to the factors that contribute to the bond durability of universal adhesives with various substrates; in particular, clinicians should be aware of their chemical bonding potential.

doi: http://dx.doi.org/10.2341/15-353-L

Microleakage and Shear Bond Strength of Composite Restorations Under Cycling Conditions

RF Zanatta • M Lungova • AB Borges • CRG Torres • H-G Sydow • A Wiegand

Clinical Relevance: The performance of composite restorations can be affected by frequent acid attacks.

doi: http://dx.doi.org/10.2341/16-132-L

Biological Effects of Provisional Resin Materials on Human Dental Pulp Stem Cells

S-K Jun • C Mahapatra • H-H Lee • H-W Kim • J-H Lee

Clinical Relevance: Possible cytotoxicity from chemical-activated provisional resin materials should be considered to avoid pulp tissue injury, particularly in extensively prepared teeth.

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Effect of Adhesive Restoration and Bleaching Technique on the Concentration of Hydrogen Peroxide In the Pulp Chamber

V Cavalli • BG Silva • SB Berger • G Abuna • FC Marson • CPM Tabchoury • M Giannini

Clinical Relevance

Hydrogen peroxide is able to diffuse into the pulp chamber even in the absence of enamel restorations. Silorane adhesive restorations allow a higher amount of hydrogen peroxide into the pulp chamber, compared to non-restored teeth.

SUMMARY

This study aimed to quantify the concentration of hydrogen peroxide into the pulp chamber in the presence or absence of adhesive enamel restorations and to analyze the resin-dentin interface of bleached groups. Bovine incisors (120) were randomly divided into three groups according to enamel treatment (n=40 each): (1) enamel without restoration (control); (2) enamel cavities (3 mm diameter × 1.5 mm depth) restored with a silorane-based (SB) system; or (3) enamel cavities (3 mm diameter × 1.5 mm depth) restored with a dimethacrylate-based (DB) system. Restorations were thermocycled, and all groups were submitted to one application of 35% hydrogen peroxide (HP) agent for 45 minutes and subjected to four light activation methods (n=10 each): without light, light-emitting diode (LED), LED/diode laser, or halogen light. Acetate buffer solution was placed into the pulp chamber before bleaching, and this solution was collected to spectrophotometrically determine the concentration of HP that reached the pulp chamber after bleaching. Rhodamine B was added to the HP

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agent and applied on additional enamel samples of each group for 24 hours. Samples were sectioned mesiodistally, and the bleaching agent was traced using confocal microscopy. According to two-way analysis of variance and Tukey test (α=0.05), the HP concentration in the pulp chamber of the control group was significantly lower than that of the SB group ($p<0.05$), regardless of light activation. No differences were observed between DB and SB groups and between control and DB groups, except for the DB halogen light activated group, which exhibited higher HP intrapulpal concentration ($p<0.05$). Confocal microscopy exhibited HP diffusion through the interface of the SB and DB restored groups as well as enamel prisms in the control group. The SB restorative system increased the HP diffusion into the pulp chamber, but HP was able to diffuse even in the absence of enamel restorations.

INTRODUCTION

Vital bleaching is believed to be a safe procedure; however, in the past few years the adverse effects of hydrogen peroxide (HP) have been revealed, particularly on the enamel surfaces. Investigations have disclosed changes on enamel morphology, an increase of enamel roughness and porosity,[1-3] and a decrease of enamel microhardness,[4,5] enamel cohesive strength,[6] and bond strength of adhesive restorations to bleached enamel.[7] Although the findings can be conflicting,[8] a decrease of enamel mineral content has also been observed; however, it could be controlled by incorporating specific concentrations of calcium and fluoride in bleaching agents.[5,9]

The mechanism of tooth bleaching is not completely elucidated; however, it is assumed that HP, the active element of bleaching agents, can form a number of different active oxygen species depending on the reaction conditions (temperature, pH, light, and presence of transition metals). Free radicals, such as hydroxyl, attack organic compounds (chromophores) that possess extended conjugated chains of alternating single or double bonds. Bleaching of the chromophore can occur by cleavage or oxidation of the conjugated chain.[10]

The oxidative mechanism of HP and the release of free radicals, which are unstable and able to react with the organic molecules, could be associated with the enamel morphologic changes.[10] In addition, the low molecular weight and the ability to diffuse into the pulp chamber brings concerns over the possible effects of HP in the pulp tissues.[11] Some studies indicate that even low HP concentrations easily diffuse through enamel and dentin, reaching the pulp chamber[12-14] and leading to pulp cell toxicity.[12-14] It has also been observed that the cytotoxicity is dose dependent on the concentration of the HP that reaches the pulp chamber[7,14,15] and that high HP concentrations could cause more damage to pulp cells than low HP concentrations.[16,17]

Based on the thermocatalytic theory, high-concentration in-office bleaching agents can be associated with different light sources (light-emitting diode [LED], LED/diode laser, halogen light) in order to increase the efficiency and hasten the decomposition of HP.[18] The efficiency of these lights on the bleaching agents is still debatable, although it was demonstrated that LED or laser applied to 35% HP increases the final concentration of HP in the pulp chamber.[19]

Trans-enamel-dentin penetration can be intensified by dentin exposure as a result of gingival recession, abrasion, abfraction, and erosion lesions, or by the presence of adhesive restorations.[13] Previous studies confirmed that HP diffusion is dependent on the quality of the enamel surface and that the HP intra-pulp concentration is higher in the presence of adhesive restorations[20] and dependent on the restorative material used.[15]

Adhesive systems with different formulations have been proposed to increase the longevity of composite restorations.[21] The main goal of these systems is to decrease the clinical application steps and the errors inherent in the application technique and to increase the adhesive stability within the hybrid layer.[22] Low-shrinkage composite resins share the same philosophy, as the intention is to decrease the marginal failures. Silorane-based composites are classified as a low-shrinkage material with polymerization shrinkage close to 1% by volume, while traditional bisphenol A glycidyl methacrylate (bis-GMA)–based composites present 2% to 5% of volumetric polymerization shrinkage.[21,23] The silorane monomer is obtained by the reaction of oxirane and siloxane hydrophobic monomers, in which the oxirane and siloxane open up during polymerization to bond to other monomers. Polymerization of the silorane is promoted by the opening of the oxirane rings that cause volumetric expansion, which tends to compensate for polymerization shrinkage.[24] But even the possible advantages of this system may not prevent HP diffusion to

the pulp chamber when a bleaching agent is applied to restored teeth.

Because more information is necessary to understand the adverse effects of HP penetration into the pulp chamber, the aim of this study was to evaluate the intra-pulp concentration of light-activated HP applied on enamel restored with different restorative systems. The null hypothesis tested was that there is no difference in the intra-pulp concentration of HP, light-activated or not, regardless of the HP application on enamel, with or without adhesive restorations.

METHODS AND MATERIALS

Experimental Design

The experimental units (120 bovine incisors) were submitted to the factors under study (n=10):

1. *Enamel treatment* (three levels): control (without enamel restoration), silorane-based (SB) restoration (Silorane System Adhesive/Filtek Silorane P90, 3M ESPE, St Paul, MN, USA), and dimethacrylate-based (DB) restoration (Scotchbond Universal/Filtek Supreme, 3M ESPE).
2. *Light-activation methods* (four levels): control (without light-activation), LED, LED/diode laser, and halogen quartz-tungsten light.

Samples were treated with a high-concentration bleaching agent (HP – 35% hydrogen peroxide, Whiteness HP Maxx, FGM, Dental Products, Joinville, Brazil). The optical density of the solution was determined spectrophotometrically and converted into micrograms equivalent to the HP. The adhesive interface was observed by confocal laser scanning microscopy.

Sample Preparation, Group Division, and Bleaching Treatment

One hundred twenty extracted bovine incisors with standardized crown dimensions were selected. After cleaning, the teeth were stored in a 0.1% thymol solution at 4°C for 30 days. In order to select those without surface defects, all teeth were examined under a stereomicroscope. The roots were cut with diamond discs (KG Sorensen, Barueri, Brazil) up to 2 mm below the cementoenamel junction and then the roots were discarded. The pulp tissue was removed using files (Hedstrom files, Maillefer Dentsply, Ballaigues, Switzerland) and then the pulp chamber was washed with distilled water. The cervical pulp orifice was widened with a round bur (No. 1016 HL, KG Sorensen) to allow for the placement of the acetate buffer solution into the pulp chamber. The total thickness (enamel and dentin) of the buccal side was measured with a caliper (Golgran, São Paulo, Brazil) and standardized to 3.5 mm. Teeth were randomly assigned to the experimental groups described above.

Enamel cavities (3 mm in diameter and 1.5 mm deep) were prepared in the buccal surface of the crowns with diamond burs (No. 3053 and No. 3017, KG Sorensen) in a standard cavity device. The adhesive systems and composites were applied according to the manufacturer's instructions (Table 1) and light-activated for 20 seconds (Valo LED, Ultradent Products Inc, South Jordan, UT, USA) with an irradiance of 800 mW/cm^2. Restorations were polished with a sequence of four sandpaper discs (Sof-Lex, 3M ESPE): coarse, 100 μm; medium, 29 μm; fine, 14 μm; and superfine, 5 μm. Each was used for 15 seconds in a single direction. At each disc exchange, the composite surface was washed and air-dried for 5 seconds; polishing discs were discarded after a single use.

Thermal Cycling

To age the bonded interface, samples of all groups were submitted to 5000 thermal cycles (MCT2 – AMM, São Paulo, Brazil) in deionized water baths at 5° to 55°C ± 1°C. For both the control and restored groups, 24 hours after the thermal-cycling procedure two coats of nail varnish (Revlon Inc, New York, NY, USA) were applied up to 2 mm around the bonded interface, leaving a standard exposed enamel area of 16.6 mm^2 for application of the bleaching agents. Teeth were fixed vertically in individual vials with the pulp chamber opening in the upper position to allow access to the acetate buffer inserted within the chamber.

Bleaching Procedure

The teeth, with or without restorations, were submitted to a single 45-minute bleaching application of a 35% HP agent (Whiteness HP Maxx, FGM; Table 1) and subjected to one of four light-activation methods (n = 10 each):

1. Enamel without restoration and bleaching without light activation
2. Enamel without restoration and bleaching combined with LED light
3. Enamel without restoration and bleaching combined with diode laser light
4. Enamel without restoration and bleaching combined with halogen light

Table 1: *Commercial Name and Manufacturer for the Materials Used, Composition, and Manufacturer Directions*[a]

Commercial Names and Manufacturers	Composition	Manufacturer Directions
Bleaching agent		
Whiteness HP Maxx *(FGM Dental products, Joinville, SC, Brazil)*	35% HP (after mixture), thickener, pigment, neutralizing agents, glycol, distilled water	After mixture, the agent is applied on the surface for 15 minutes and stirred 3-4 times to promote oxygen release; repeat twice. Light sources can be used to accelerate bleaching.
Dental Restoratives		
Filtek Z350 XT (3M/ESPE, St Paul, MN, USA)	Bis-GMA, UDMA, TEGDMA, Bis-EMA particles of silica and zircônia/silane, BHT, photoiniciator system, and pigments.	Composite increments of 2 mm
Filtek Low Shrinkage Posterior P90 - Silorane (3M/ESPE)	Particles of quartz and silica/silane, yttrium fluoride, 3,4-epoxy cyclohexylethylcyclopolymethylsiloxane, bis-3,4-poxycyclohexylethyl phenylmethylsilane,initiator system: camphorquinone and iodonium salt (donator of electrons), stabilizers, and pigments	Composite increments of 2 mm
Silorane Adhesive system – Self-etch primer (3M/ESPE)	Phosphate methacrylates, copolymer of Vitrebond, Bis-GMA, HEMA, water, ethanol, particles of silica treated with silane, initiators, and stabilizers	Dry enamel and apply the self-etching primer with moderate friction for 10 seconds, air dry for 5 seconds, and light cure for 10 seconds. Apply the adhesive and light cure for 10 seconds.
Silorane Adhesive system – Bond (3M/ESPE)	Hydrophobic dimethacrylate, phosphate methacrylates, TEGDMA, particles of silica treated with silane, initiators, and stabilizers	
Scothbond Universal (3M/ESPE)	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, water, initiators, and silante	Enamel acid etching for 15 seconds; wash and dry; apply the adhesive for 20 seconds, air-dry for 5 seconds, and light cure for 10 seconds.

[a] *Source: MSDS data sheet*
Abbreviations: BHT, butyl hydroxy toluene; Bis-EMA, bisphenol A ethoxylated dimethacrylate; Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, hydroxyethylene glycol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate;UDMA, urethane dimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate

5. Enamel restored with SB system and bleaching without light activation
6. Enamel restored with SB system and bleaching combined with LED light
7. Enamel restored with SB system and bleaching combined with diode laser light
8. Enamel restored with SB system and bleaching combined with halogen light
9. Enamel restored with DB system and bleaching without light activation
10. Enamel restored with DB system and bleaching combined with LED light
11. Enamel restored with DB system and bleaching combined with diode laser light
12. Enamel restored with DB system and bleaching combined with halogen light

The bleaching agent was weighed (0.01 g), applied on the exposed enamel area and then light-activated, according to the manufacturer's instructions (Table 2).

Concentration of Hydrogen Peroxide Into the Pulp Chamber

Before the bleaching treatment, the pulp chambers were individually dried and filled with 150 μL of 2 mol/L acetate buffer (pH 4.5). The acetate buffer solution was applied to stabilize the HP that penetrates into the pulp chamber throughout the bleaching. After bleaching, the acetate buffer was removed and the solution was transferred to a glass test tube. The pulp chamber of each tooth was filled for a second time with 150 μL acetate buffer for 1 minute, and this solution was placed in the same glass tube. In addition, distilled water (2650 μL), Leuco Crystal Violet (100 μL of 0.5 mg/mL; Sigma-Aldrich, St Louis, MO, USA), and horseradish peroxidase (50 μL of 1 mg/mL; Sigma-Aldrich) were also added to each tube, according to the method described by Berger and others.[25]

This solution produced a blue color, and the optical density obtained in the tubes was measured in a

Table 2: *Light Sources, Product Features, and Application Protocol*[a]

Light Sources	Commercial Names	Characteristics	Application Protocol
LED light	Valo (Ultradent Products Inc, South Jordan, UT, USA)	Wavelength: 395-480 m Light intensity: 790 mW/cm^2	The bleaching gel remains 1 minute without agitation and is light irradiated for 2 minutes. The procedure is repeated three times with 1-minute interval among irradiations.
LED/laser diode	Whitening Laser Light Plus (DMC Equipment, São Carlos, Brazil)	LED wavelength: 470 ηm Infrared diode laser wave length (3): 810 ηm and power of 0.2 W	The bleaching gel remains 1 minute on the surface without agitation and is light irradiated for 2 minutes. The procedure is repeated three times with 1-minute interval among irradiations.
Halogen light	Optilux 501 (Demetron/Kerr, Danbury, CT, USA)	Wavelength: 560 ηm Light intensity: 600-800 mW/cm^2	The bleaching agent is applied on the surface for 2 minutes and irradiated for 30 seconds. The irradiation is repeated three times with a 2-minute interval.

[a] *Source: MSDS data sheet*
Abbreviation: LED, light-emitting diode.

spectrophotometer (DU 800, Beckman Coulter Inc, Brea, CA, USA) at a wavelength of 596 nm. A standard curve of known HP concentrations was obtained to convert the optical density values of each specimen into microgram (μg) equivalents of HP/mL of solution. The values were then converted into micrograms per milliliter. Due to the chemical instability of HP,[26] a second standard curve of known HP concentrations was obtained to determine the real concentration of the commercially available bleaching agent. This concentration was measured for the stoichiometric calculation.

Laser Scanning Confocal Fluorescence Microscopy (LSCFM)

To determine the integrity of the adhesive interface, three additional samples were prepared and observed using LSCFM. Rhodamine B (0.1 mM, Sigma-Aldrich) was added to the bleaching agents and the agents were placed in contact with the enamel surface at the recommended bleaching time. After bleaching, teeth were sectioned (with oil lubrication) at the central area of the restorations (Isomet 1000, Buehler, Lake Bluff, IL, USA) and the exposed inner interfaces were ground and polished (polishing machine, Ecomet 3000, Buehler) with abrasive paper (No. 400, No. 600, and No. 1200) and diamond pastes (0.3 and 0.1 μm, Metaldi Supreme, Buehler). The samples were sonically cleaned to remove polishing residues, and the interface area was analyzed using LSCFM (TCS SP5AOBS, Leica Microsystems CMS GmbH, Wetzlar, Germany), then scanned by an argon laser with a wavelength of 529 nm. The groups submitted to light-activation methods (LED, LED/diode laser, and halogen light) were not observed by LSCFM, as rhodamine B is sensitive to light variations, a factor that could compromise the results. Figure 1 schematically depicts the methodologic procedures.

Statistical Analyses

The exploratory data analysis of the results was submitted to the software Proc Lab (SAS 9.0, SAS Institute, Cary, NC, USA). The normal distribution and homoscedasticity of the values were verified and a parametric analysis was performed. The concentration of HP that reached the pulp chamber (μg/mL) was submitted to two-way analysis of variance (ANOVA) (enamel treatment × light activation) and Tukey test (pre-set alpha of 0.05).

RESULTS

A summary of the HP concentration in the pulp chamber (μg/mL) is shown in Table 3. Two-way ANOVA and Tukey test indicated that the HP concentration in the pulp chamber of the group without enamel restorations (control) was significantly lower than that of the groups restored with the SB system, regardless of the light activation method used ($p<0.00001$). The same analyses indicated that the intrachamber HP concentration of enamel restored with the DB system was significantly higher than that of the control group when HP was combined with halogen light activation

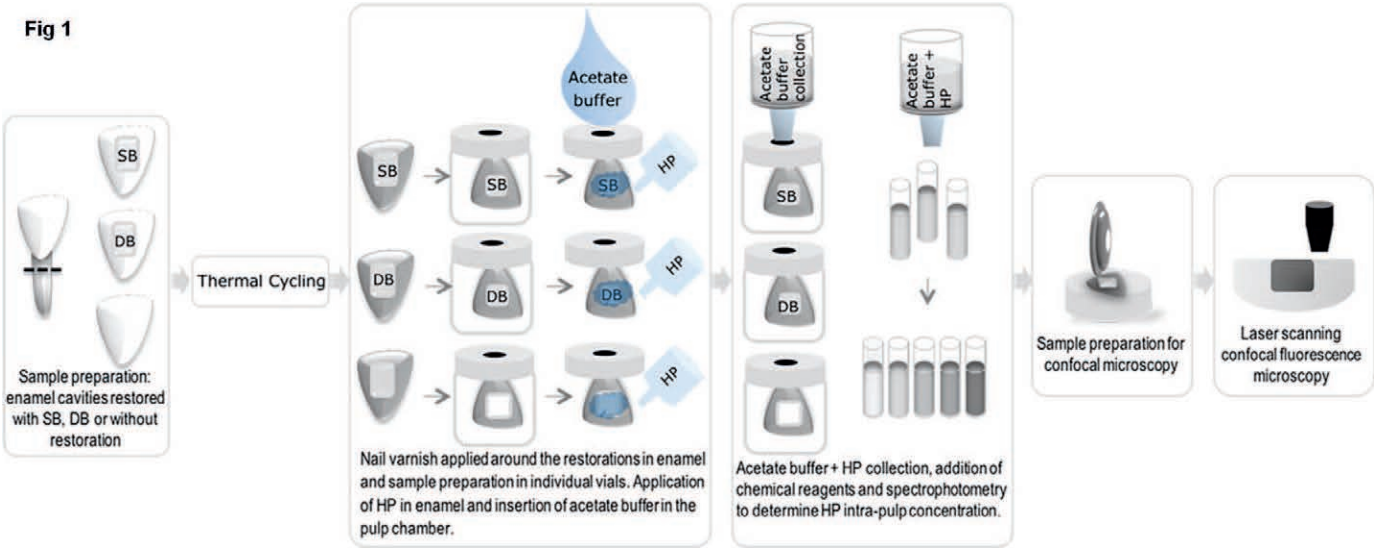

Figure 1. *Schematic representation of the methodological procedures.*

($p<0.05$). Finally, no differences were observed comparing the data from the two groups with enamel restorations (SB and DB, $p>0.05$), regardless of the light-activation method used.

The LSCFM images of the HP penetration in the enamel/dentin interface and/or at the adhesive interface are represented in Figure 2A-C. Figure 2A represents the control group (enamel without restoration). Figures 2B and C represent the enamel with SB and DB adhesive restorations, respectively. In Figure 2A, the greatest concentration of rhodamine B is observed in the aprismatic enamel, whereas in Figures 2B and C, the bleaching agent penetrated the interface, reaching the dentin surrounding the adhesive interface.

DISCUSSION

The results of this study indicate that there were no differences in the intra-pulp concentration of HP between the groups with adhesive restorations (silorane or dimethacrylate). However, the intra-pulp concentration of HP of enamel without a restoration (control) was significantly lower compared with that of the silorane-treated group. Therefore, the null hypothesis was rejected because the SB adhesive restorations exhibited higher HP intra-pulp concentration than the control group, regardless of the light activation method used.

The permeability of the dental structure associated with the ability of the transenamel/dentin diffusion of the bleaching agents has become the focus of a number of research studies that evaluated the cytotoxic effects of HP free radicals that reach the pulp. In general, the studies evaluate the effects of bleaching agents on cultured odontoblast-like MDPC-23 cells.[27,28] According to some of these evaluations, HP toxicity is dose dependent, but even the lowest peroxide concentrations could promote

Table 3: *Mean and Standard Deviation of Hydrogen Peroxide (HP) Concentration in the Pulp Chamber (μg/mL) After 35% HP Treatments (Light Activated or Not) on Enamel Without (Control) and With Dimethacrylate-Based (DB) and Silorane-Based (SB) Adhesive Restorations*[a]

Groups	Control (Without Restoration)	DB Restorative System	SB Restorative System
35% HP	4.1 (2.4) Aa	7.9 (4.5) Aab	8.3 (4.9) Ab
35% HP + light-emitting diode light	3.2 (2.5) Aa	7.4 (2.5) Aab	10.5 (4.7) Ab
35% HP + diode laser light	3.1 (2.3) Aa	7.5 (2.7) Aab	8.8 (4.5) Ab
35% HP + halogen light	2.9 (2.4) Aa	10.6 (4.3) Ab	11.0 (4.0) Ab

[a] *Means followed by distinct letters differ statistically at 5%, according to two-way analysis of variance and Tukey test ($p<0.05$; uppercase letters for columns, lowercase letters for rows).*

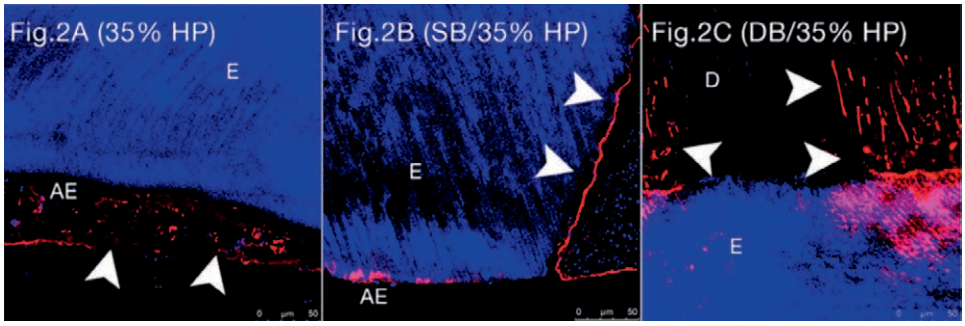

Figure 2. *Laser scanning confocal fluorescence microscopy of bleached enamel (with or without adhesive restoration). (A): In the control group (intact enamel), the arrows indicate the infiltration of rhodamine B dye (in red) and demonstrate the penetration of HP into the aprismatic enamel (AE). (B): The arrows indicate the infiltration of rhodamine B dye at the adhesive interface and dye deposition at the aprismatic enamel (AE). (C): The arrows indicate the infiltration of rhodamine B dye first in enamel (E) and, afterward, reaching dentin (D).*

pathological effects and reduce cell viability by approximately 77%.[13,14,27]

In previous *in vivo* evaluations, pulp damage was observed in human incisors after a 38% HP application on enamel for 45 minutes.[28] In another *in vivo* study, after the application of a calcium-free 35% HP agent applied three times for 15 minutes each or a single 45-minute application on enamel, both without light activation, the coronal pulp tissue exhibited partial necrosis associated with tertiary dentin deposition in five mandibular extracted incisors.[29]

The enamel/dentin permeability, time, application mode,[25] heating/light-activation,[30] quality and thickness of enamel,[15] and presence of adhesive restorations[20] are among the factors that influence HP penetration into the pulp chamber. The intra-pulp HP concentration was greater in the SB group compared with the control group; however, the DB group was similar to the control (except for halogen light activation) and the SB groups. Benetti and others[20] observed that the intra-pulp concentration of HP free radicals was greater in the presence of adhesive restorations, whereas Camargo and others[15] reported that among different restorative materials (composite resin, glass ionomer cement, and glass ionomer cement modified by resin) HP penetration into the pulp chamber was dependent on the material used and was lower for composite resin restorations. It is known that HP penetration occurs due to its low molecular weight and ability to denature proteins, which increases the ionic movement through the dental hard structure.[25] In addition, HP penetration can increase due to polymerization shrinkage that creates gaps at the enamel/resin interface and possible changes in the adhesive interface of enamel restorations promoted by the bleaching agents.[15,31,32]

In the current study, restorations were thermocycled before HP application, in order to age the interface and promote adhesive failure. Although two different adhesives were evaluated, it is noteworthy that after 5000 cycles, there were no perceptible failures in the enamel interface; however, when penetration of HP was assessed, the SB agent allowed greater HP penetration than the DB agent. The silorane adhesive is a self-etching adhesive, and according to its application technique, the manufacturer suggests excluding enamel etching. Therefore, it is possible that application of the silorane adhesive without etching of the cavosuperficial enamel contributed to the inferior performance of this system. It is postulated that a significant increase in enamel bond strength results from enamel etching before the application of this adhesive.[33,34]

In a recent study,[35] the performance of the SB restorative system was evaluated with respect to polymerization shrinkage in low (Class V) and high (Class I) C-factor cavities. According to that study and another investigation,[34] the silorane composite promoted higher polymerization shrinkage stress compared with the conventional dimethacrylate adhesive system, and the reason could be related to the monomeric composition.[35] The silorane adhesive system is composed of a hydrophilic acidic primer (SSA Primer) with a mild pH of 2.7, which promotes dentin demineralization of approximately a few hundred nanometers. The primer of the silorane system is composed of a dimethacrylate phosphate functional monomer, hydroxyethylene glycol dimethacrylate (HEMA), BIS-GMA, itaconic acid copolymer, silica, and camphorquinone dissolved in a water-alcohol solution.[34,35] The bonding resin is a hydrophobic high-viscosity agent (SSA Bond), basically composed of dimethacrylate, triethylene glycol dimethacrylate (TEGDMA), silica, camphorquinone, and function monomers.[33,35] The positive aspect of the high concentration of HEMA in the primer is that it prevents phase separation. On the other hand, HEMA increases the susceptibility of this adhesive to water sorption. Further, due to the highly hydrophobic nature of the adhesive, previous

studies have observed the presence of water between the layers of the primer and the hydrophobic adhesive resin.[33,35] Therefore, the adhesive interface may be considered the problematic link of the SB system[21,33] and possibly the reason for the higher HP intra-pulp penetration.

Some studies have evaluated the microleakage of SB and DB materials. In an *in vitro* experiment, microleakage was observed in Class I restorations using different bonding techniques for both SB and DB materials, but the SB materials showed more microleakage compared with the DB composite systems.[36] Another evaluation observed that the SB material did not provide better marginal integrity than low shrinkage methacrylate-based composites.[37] According to the authors, this microleakage could be related to the formation of an oxygen inhibition layer during the curing of silorane primer before the application of the bonding agent.[37,38] This layer formed between the cured primer and the bond was observed in micro-Raman spectroscopy as an intermediate zone of approximately 1 μm and may be the weakest zone of silorane adhesives.[38]

Another *in vitro* study[39] evaluated the effect of 30% HP on marginal integrity of adhesive restorations. It observed that although bleaching did not significantly affect the marginal integrity of the restorations, the SB composite exhibited greater microleakage in gingival margins than the DB composites tested. The authors also suggested that the micrometer intermediate zone was the weakest link of the interface and proposed that the free radicals released by HP could lead to an increase in the microleakage of SB compostites.[39]

The DB restorative system achieved intermediate penetration values, which were not statistically different from those of the control group. It is important to observe that the application of this adhesive involved selective enamel etching, which was carried out as recommended by the manufacturer. In a preceding study, the good performance of this adhesive was noted when selective enamel etching was performed even after thermal cycling.[40] As rhodamine B was added to the bleaching gel, the dye penetration shown in the laser scanning confocal fluorescence microscopy was the pathway of HP in the enamel/dentin/resin-tooth interfaces. It can be noted that the dye penetration was more noted in the presence of the adhesive interface (either SB or DB restoratives). The interface aging with thermal cycling might have caused damage at the bonded interface, which enabled the infiltration of the peroxide along with the dye.

According to the thermocatalytic principle of HP decomposition, light application (LED, LED/diode laser, halogen light) combined with in-office bleaching agents aims to accelerate the decomposition of HP.[18] This principle states that the temperature increase accelerates the release of hydroxyl radicals, so when light energy strikes the bleaching agent, a fraction is absorbed and the acquired energy is converted into heat. To increase light absorption and, as a result, increase heat conversion into energy, some agents contain specific dyes, such as carotene, which has a reddish color and increases the absorption of blue light. To increase the absorption of red or infrared light, some agents contain silica nanoparticles, which provide a bluish color to the bleaching agents.[18] However, the effectiveness of light activation of bleaching agents is still controversial.[41] Hein and others[42] observed that the application of heat and light was unable to increase *in vitro* HP decomposition, but the temperature of the agents containing carotene increased it considerably, raising concerns about pulp injuries. It has also been observed that activation of 35% HP with an LED light or LED/diode laser increases the final concentration of peroxide into the pulp chamber,[19,41] which possibly promotes cell injuries.[29] Contrarily, it has been previously observed that the application of a laser or quartz tungsten halogen light was unable to increase bleached enamel permeability; however, bleaching promoted greater permeability than unbleached enamel without light irradiation.[43]

In the present study, no differences were noted in the pulp chamber concentration of HP between the control and DB groups when exposed to different light activation sources (LED, LED/diode laser, or halogen light), except for the DB group combined with halogen light, which exhibited a greater HP concentration compared with the control group. Although only one group (DB/halogen light) presented greater HP concentration compared with the control, the authors of this study indicate that light activation of any type could be dismissed since the decomposition of the agents occurs even in the absence of light.[26]

Previous studies have noted that the penetration of high-concentration HP agents was dependent on the application time, since the longer the contact of the agent with the enamel surface, the higher the intra-pulp concentration[44] and, possibly, the greater the risk of cytotoxicity. As a consequence, reducing the application time of 35% HP has been proposed as a way to reduce the cytotoxic effects.[45,46] According

to these authors, reducing the application time would not compromise the desired whitening effect and would decrease tooth sensitivity and the adverse effects in pulp cells.[44,45]

Although a significant number of articles indicate toxicity effects in pulp cells, other studies attest that bleaching agents do not generate acute or minor toxic effects. Vongsavan and Matthews[47] maintain that *in vitro* studies typically show greater penetration of peroxides than those performed under *in vivo* conditions. These authors verified that dye penetration into the pulp chamber was lower in *in vivo* than *in vitro* conditions because pulpal pressure and the dentinal fluids (a mixture of albumin, transferrin, tenascin, and proteoglycans) that flow inside the tubules from the pulp to the outer dentin surface prevent the diffusion of fluids and other substances into the pulp chamber.[47] Therefore, the use of extracted teeth without pulpal pressure and dentinal fluids probably allows greater intra-pulp penetration than in clinical situations. Still, one should consider that the pulp tissue has a self-protection system against damages caused by HP by means of a production of enzymes, such as peroxidase and catalase, which promote the molecular breakdown of the products of the HP reaction.

The immediate and most common clinical consequence of pulp tissue alteration is dental hypersensitivity[45] that, although transient, still causes discomfort to the patient and uncertainties regarding the long-term consequences of bleaching. Thus, *in vivo* long-term studies evaluating HP pulp penetration would clarify the magnitude of undesirable clinical consequences.

CONCLUSION

The bleaching agent tested (35% HP) is able to reach the pulp chamber in the presence or absence of enamel adhesive restorations. Among the groups, it could be observed that the HP intra-pulp concentration was greater in the presence of an SB adhesive restoration compared with intact enamel (control, ie, without adhesive restoration). Different light sources did not influence the HP intra-pulp penetration for the control group and enamel with an SB restorative system.

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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 Campinas, Piracicaba Dental School.

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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Fifteen-year Clinical Follow-up of Restoration of Extensive Cervical Resorption in a Maxillary Central Incisor

EG Reston • RPR Bueno • LQ Closs • J Zettermann

Clinical Relevance

This multidisciplinary approach allowed us to maintain the tooth and bone height, contributing to the patient's facial esthetics and obviating the need for more complex and invasive procedures, along with the psychological aspect of maintaining the tooth in the dental arch.

SUMMARY

Internal bleaching in endodontically treated teeth requires care and protection to prevent harm to the periodontal ligament due to peroxide and may result in external root resorption. There is a myriad of treatment options when this occurs, such as monitoring, extraction, and subsequent rehabilitation with implants or fixed prosthodontics. In some cases, such as the one described here, a conservative attempt to maintain the tooth as a single structure can be made by sealing the resorptive defect. In the present case, we show a multidisciplinary approach where orthodontics, periodontics, and restorative dentistry were involved in treating the maxillary right central incisor (#8) of a 65-year-old patient with extensive cervical resorption, whose chief complaint was esthetics. The proposed treatment was extrusion of the tooth followed by curettage and restoration of the defect with glass ionomer cement. The patient has been followed for 15 years with no signs of recurrence, maintenance of periodontal health, and patient satisfaction with the esthetic outcome.

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DESCRIPTION OF THE TECHNIQUE

A 65-year-old patient sought dental treatment mainly due to esthetic concerns, and the initial periodontal evaluation indicated the presence of a subgingival cavity in the root surface of tooth #8 (maxillary right central incisor). The patient's medical history revealed internal bleaching of tooth #8 six years before. A periapical radiograph showed extensive structural loss directly related to external cervical resorption (Figures 1 and 2). Conventional treatment would involve tooth extraction and placement of an osseointegrated implant. However, at that time, implants did not have the esthetic features and dimensions that they do today.[1] Moreover, the technique should be mastered, and

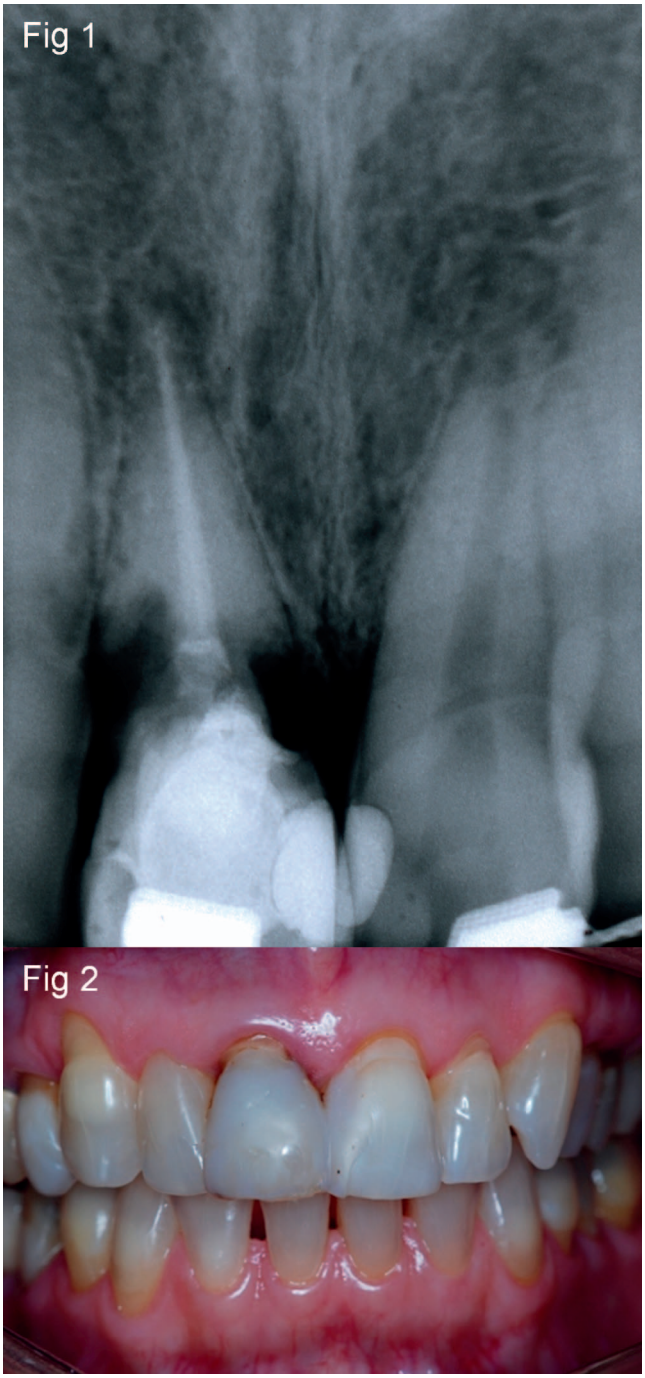

Figure 1. *Initial periapical radiograph.*
Figure 2. *Initial clinical aspect.*

gingival recontouring would be required in tooth #9 (the maxillary left central incisor). Fixed prosthodontics was discarded by the patient due to the risk of damage to adjacent tooth structures. The gingival smile line and maintenance of the existing harmony were considered compromising factors.

A multidisciplinary approach to the case allowed us to offer the patient an alternative treatment. The patient was aware of the risk of possible failure and, consequently, of the need to return to one of the options initially considered in case of failure. Professionals from the fields of orthodontics, periodontics, and restorative dentistry were involved. The procedures are described below in chronological order.

Tooth Traction/Extrusion

The lesion was located approximately 3 mm below the crestal bone, and tooth extrusion was therefore proposed with the use of orthodontic traction. Using the straight wire technique, 0.022-inch slot brackets (Ormco, BR Amersfoort, The Netherlands) were bonded to the six maxillary anterior teeth. The adjacent central incisor served as anchorage, and a sequence of nickel-titanium (NiTi) and stainless steel wires was used for traction. Within 80 days, an extrusion of 3 mm was achieved (Figure 3). The device was kept passive for 3 months until complete tissue reorganization and periodontal bone healing[2-5] (Figure 4).

Surgical Access

The periodontal access technique was selected, aiming to preserve the alveolar crest and gingival tissue as much as possible. The gingiva was detached, and a small portion of the crestal bone was removed near the cavity, exposing its margins.[5-7]

Restoration of the Resorptive Defect

The cavity was restored trans-surgically. After visual access to the area, the affected portion was excavated and cleaned with periodontal curettes. Moisture control is a challenge in trans-surgical restoration, and based on the literature, a light-cured glass ionomer cement (Vitremer, 3M ESPE, St Paul, MN, USA) was selected and injected into the cavity with a Centrix syringe.[6,8-15] After the initial setting, the finishing process of the glass ionomer cement was performed and the area was then sutured.

To improve esthetics, a direct composite veneer was placed. The use of ceramic restoration was not considered because of the overall condition of the tooth, including a weakened structure and uncertain prognosis. The composite restoration met the patient's esthetic needs at the time and has been maintained since placement.

The patient returned for evaluation and suture removal seven days after the procedure.

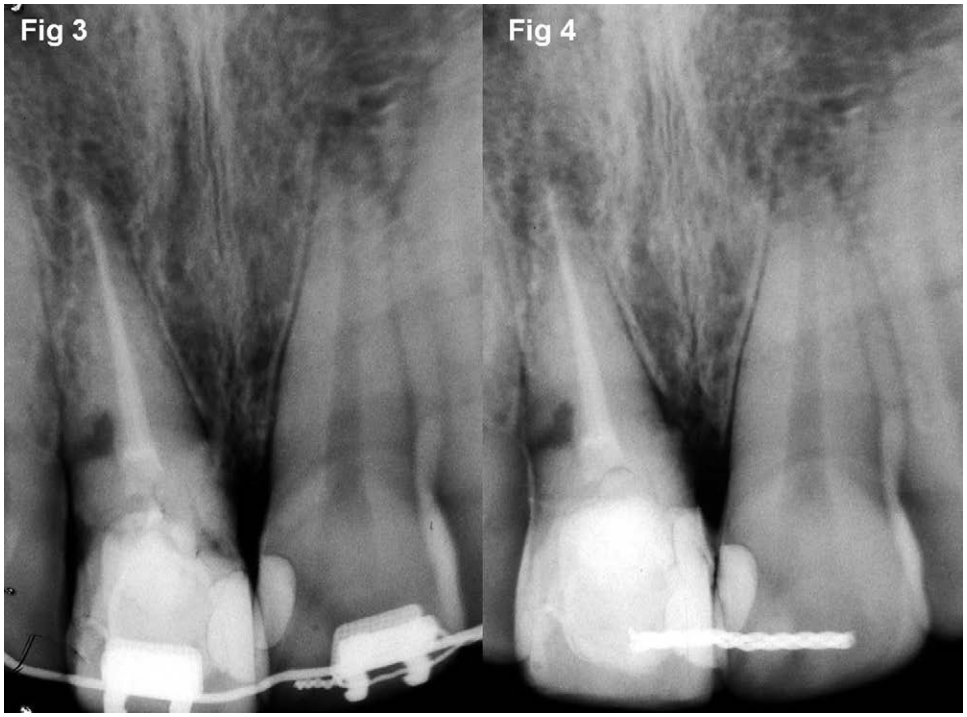

Figure 3. *Orthodontic extrusion and GIC restoration.*
Figure 4. *Fixation after extrusion.*

Since then, the patient has been followed radiographically every six months with no evidence of recurrence of resorption thus far (Figures 5 and 6).

POTENTIAL PROBLEMS

During the course of treatment, unpredictable outcomes may occur. Orthodontic tooth extrusion may result in crown-root fracture, as well as in the inability to expose the resorbed area. The necessary exposure may result in excessive bone removal during surgical access, compromising the final esthetic result. In addition, the filling of the cavity may be incomplete and fail to prevent disease progression.[16]

A prefabricated post was placed eight months after the defect was sealed. Although it does not reinforce the structure, it could possibly increase retention. The authors understand that the post placed was short, and could be longer, but not wider, to enhance retention without transferring stresses to the weakened walls which were reinforced by glass ionomer cement as a dentin replacement.

ADVANTAGES AND DISADVANTAGES

Maintenance of tooth structure, along with its psychological aspect, and stabilization of bone height can be considered the main advantages of the presented technique. Furthermore, there was no impairment of the already established esthetics, which could occur in case of tooth extraction, the healing process, and implant placement.

The main concern is the unpredictable longitudinal behavior and therefore inability to ensure the longevity of the procedure to the patient. The technique may appear to be more complex than implant placement, but when performed by trained professionals, the success rate can be improved. Additionally, at the time this case was treated, the knowledge of dental implants was not as developed as it is today.

Regulatory Statement

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

Conflict of Interest

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

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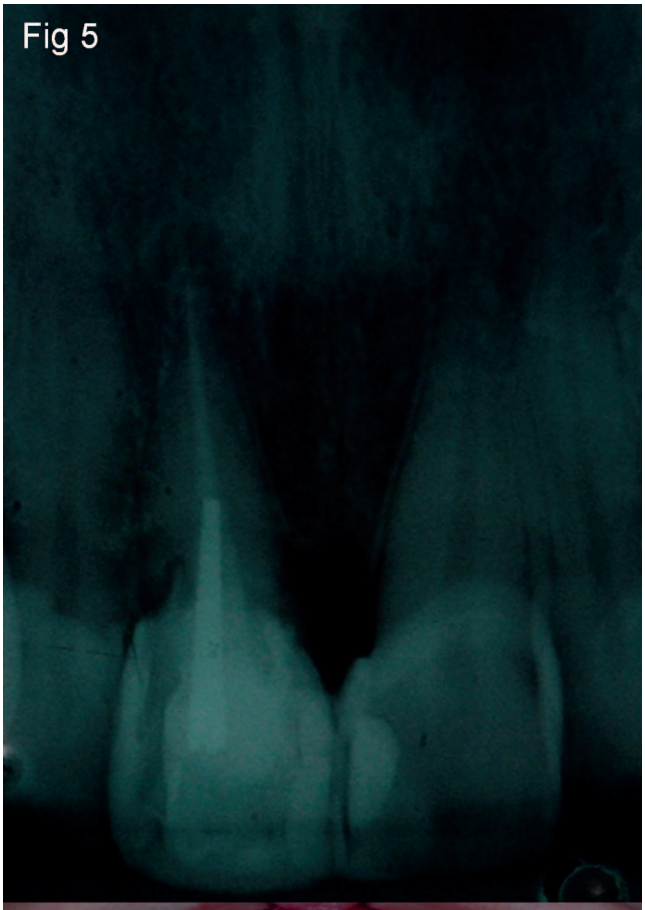

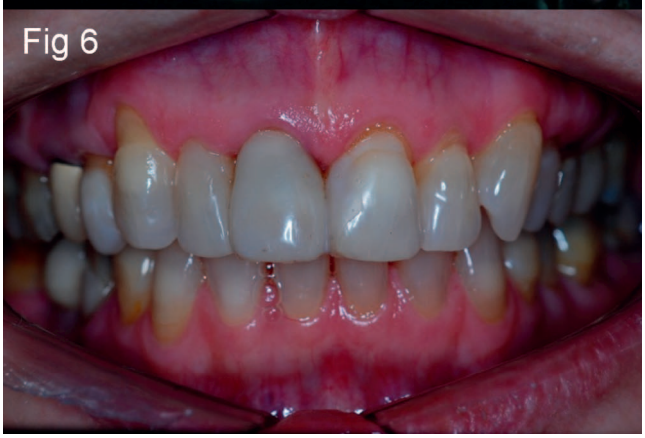

Figure 5. *Fifteen-year follow-up radiograph.*
Figure 6. *Fifteen-year follow-up clinical aspect, after esthetic direct resin veneer.*

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Interfacial Characteristics and Bond Durability of Universal Adhesive to Various Substrates

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Clinical Relevance

Clinicians should follow the procedures associated with universal adhesives to modify the interfacial characteristics carefully and pay attention to the factors that contribute to the bond durability of universal adhesives with various substrates; in particular, clinicians should be aware of their chemical bonding potential.

SUMMARY

Objective: This study investigated the interfacial characteristics and bond durability of universal adhesives to various substrates.

Methods and Materials: Two universal adhesives were used: 1) Scotchbond Universal and 2) G-Premio Bond. The substrates used were bovine enamel and dentin with or without phosphoric acid etching, resin composite, lithium disilicate and leucite-reinforced glass ceramics, zirconia, and metal alloys. The surface free energy and the parameters of various substrates and of substrates treated by adhesive after light irradiation were determined by measuring the contact angles of three test liquids. Resin composite was bonded to the various substrates to determine shear bond strength after 24 hours water storage and 10,000 thermal cycles. A one-way analysis of variance (ANOVA) and the Tukey post hoc test were used for the surface free energy data, and a two-way ANOVA and the Tukey post hoc test were used for analysis of shear bond strength data (α=0.05).

Results: The interfacial characteristics of the various substrates show significant differences depending on the type of substrate, but the interfacial characteristics of substrate treated by adhesive after light irradiation did not show any significant differences regardless of the substrate used. The bond durability of two universal adhesives to various substrates dif-

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fers depending on the type of substrate and the adhesive.

Conclusions: The results of this study suggest that universal adhesives modify the interfacial characteristics of a wide range of substrates and create a consistent surface, but the bond durability of universal adhesive to various substrates differs depending on the type of substrate and the adhesive.

INTRODUCTION

The introduction of new-generation adhesive systems has aimed at reducing technique sensitivity and the number of clinical steps required for adhesion.[1] There has been a trend toward the use of less time-consuming options, such as single-step self-etch adhesives.[2] Continuing this trend, universal adhesives have recently been introduced to the profession.[3] Universal adhesives are designed to bond to tooth structures via the total-, self-, or selective-etch technique.[4] In addition, some universal adhesives are also capable of bonding to various substrates, including resin composite, glass ceramics, zirconia, and metal alloys, with no need for additional primers.[5] The versatility offered by universal adhesives provides for a new, simplified approach to bonding between resins and various substrates.[6] However, because of the recent introduction of universal adhesives, little information is currently available about the bond durability of universal adhesives to various substrates.

The surface modification of various substrates by universal adhesive is important for bonding with resin composite because the surface properties of restorative materials differ.[7] Previous studies[8,9] have shown that the surface modification and coating capacity of adhesives can be analyzed on the fundamentals of interface science. The interfacial characteristics of substrates treated by adhesives, including wetting ability, polarization, and hydrophilicity, may be measured in terms of the surface free energy.[10,11] Adhesion requires intimate contact at both the substrate-adhesive and adhesive-restorative interfaces in order to form tight and durable connections.[12] Since the interfacial characteristics of the substrates that compose the adhesive interface are different depending on their compositions, understanding of the interfacial characteristics of substrates and substrate treated by adhesive is essential to understanding and promoting intimate contact among substrate, adhesive, and restorative materials.[13] Thus, analysis of the interfacial characteristics of a wide range of substrates treated by universal adhesives may provide novel insight into the basis of the bond durability of universal adhesive with various substrates.

Evaluation of bond durability is important, since the stability of the bond between the adhesive and substrates is related to the long-term clinical success of restorations.[14] Although the most reliable conclusions about the performance of adhesives in the oral environment are derived from long-term clinical trials, long-term aqueous storage of the bonded specimen or subjecting it to thermal cycling may provide valuable information about bond durability.[15] A thermal cycling test is the process of subjecting bonded specimens to cyclic temperature changes through water immersion in order to simulate intraoral conditions.[16] A previous study[17] established that 10,000 thermal cycles (TC) correspond to one year of clinical function of restorations, and this estimate is based on the hypothesis that such cycles might occur 20 to 50 times a day. Universal adhesives differ from the current self-etch adhesives in the incorporation of monomers that are capable of modifying surfaces and producing chemical bonding to the various substrates.[18] A commonly used monomer is 10-methacryloyloxydecyl dihydrogen phosphate (MDP), which helps bond not only tooth substrates but also metal oxides,[5,7] and some universal adhesives include silane to bond with glass ceramics[6] or sulfur-containing monomers to improve bonding with noble metals.[19] It is postulated that this incorporation may increase the bond durability of universal adhesives to various substrates.

The purpose of this laboratory study was to investigate the influence of universal adhesive on the interfacial characteristics of various substrates by measuring changes in surface free energy and the parameters. In addition, the bond durability of universal adhesives to various substrates was determined to assess the effects of the surface modifications. The null hypotheses to be tested were the following: 1) The interfacial characteristics of various substrates would not be influenced by treatment with universal adhesives; and 2) There would not be differences in the bond durability of universal adhesives to various substrates.

METHODS AND MATERIALS

Adhesive Systems

Two universal adhesives were used in this laboratory investigation: 1) Scotchbond Universal (SU; 3M ESPE, St Paul, MN, USA) and 2) G-Premio Bond (GB; GC, Tokyo, Japan). The adhesives and associ-

Table 1: *Materials Used in This Study*

Materials (Lot No.)	Type of Material (Code)	Main Components	Manufacturer
Scotchbond Universal (566724)	Universal adhesive (SU)	MDP, Bis-GMA, HEMA, Vitrebond copolymer, polyethylene glycol, water, initiator, colloidal silica, silane	3M ESPE, St Paul, MN, USA
G-Premio Bond (541424)	Universal adhesive (GB)	MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, initiator, silica	GC, Tokyo, Japan
Clearfil AP-X (1312131)	Resin composite (RC)	Bis-GMA, TEGDMA, silanated barium filler, silanated colloidal silica, DL-camphorquinone, catalysts, accelerators, pigments, others	Kuraray Noritake Dental, Tokyo, Japan
IPS e.max CAD (P23546)	Lithium disilicate glass ceramic (LD)	SiO_2, Li_2O, K_2O, P_2O_5, ZrO_2, ZnO, other oxides, pigments	Ivoclar Vivadent, Schaan, Lichtenstein
IPS empress CAD (T43858)	Leucite-reinforced glass ceramic (LR)	SiO_2, Al_2O_3, K_2O, Na_2O, other oxides, pigments	Ivoclar Vivadent
IPS e.max ZirCAD (T22482)	Zirconia (ZR)	SiO_2, Al_2O_3, K_2O, Na_2O, other oxides, pigments	Ivoclar Vivadent
Casting Gold M.C. type III (1011741)	Type III gold alloy (GA)	Au, Cu, Ag, Pd, others	GC
Castwell M.C. 12 (1312041)	Au-Ag-Pd alloy (AP)	Au, Ag, Pd, Cu, Au, others	GC
Ultra-Etch (N017)	Phosphoric acid pre-etching agent	35% Phosphoric acid	Ultradent Products, South Jordan, UT, USA
Ceramic Primer II (1402101)	Silane coupling agent	Silane, MDP, ethanol	GC

Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; 4-MET, 4-methacryloyloxyethyl trimellitate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; MEPS, methacryloyloxyalkyl thiophosphate methylmethacrylate; TEGDMA, triethylene glycol dimethacrylate.

ated lot numbers and components are shown in Table 1. Ultra-Etch (Ultradent Products, South Jordan, UT, USA) was used as a 35% phosphoric acid pre-etching agent. According to the manufacturers' instructions, SU does not require a silane coupling treatment to glass ceramics, but GB does require a silane coupling treatment (Ceramic Primer II, GC) to bond adhesive to glass ceramics.

Bonding Substrates

The substrates for bonding with universal adhesives (Table 1) were as follows: 1) bovine enamel and dentin, 2) resin composite: Clearfil AP-X (RC; Kuraray Noritake Dental, Tokyo, Japan), 3) lithium disilicate glass ceramic: IPS e.max CAD (LD; Ivoclar Vivadent, Schaan, Liechtenstein), 4) leucite-reinforced glass ceramic: IPS empress CAD (LR; Ivoclar Vivadent), 5) zirconia: IPS e.max ZirCAD (ZR; Ivoclar Vivadent), and 6) metal alloys: Casting Gold M.C. type III (GA, GC) and Castwell M.C. 12 (AP, GC).

Specimen Preparation

Mandibular incisors extracted from two- to three-year-old cattle and stored frozen (−20°C) for up to two weeks were used. After removing the roots using a water-cooled precision diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA), the pulps were removed, and the pulp chamber of each tooth was filled with cotton to prevent penetration of the embedding media. After ultrasonic cleaning for 30 seconds in distilled water to remove excess debris, the surfaces were washed and dried with oil-free compressed air. The labial surfaces were ground with wet #180-grit silicon carbide (SiC) paper to create flat enamel and dentin surfaces.

LD, LR, and ZR plates were cut from CAD/CAM ceramic blocks using a water-cooled precision diamond saw to produce specimens that were $10 \times 10 \times 2$ mm thick. All of the ceramic plates were crystallized in a ceramic furnace (Programat S1, Ivoclar Vivadent) according to the manufacturers' instructions.

Metal disks, 10 mm in diameter and 2 mm in thickness, with an attached loop were fabricated with the flat surface of each disk perpendicular to the loop and cast in GA and AP according to the manufacturers' instructions.

Each specimen was then mounted in self-curing acrylic resin (Tray Resin II, Shofu, Kyoto, Japan) to expose the flattened area and placed under tap water to reduce the temperature rise caused by the exothermic polymerization reaction of the acrylic resin. The surfaces of various substrates were ground with #320-, #600-, #1200-, and #2000-grit

SiC paper. These surfaces were then washed and dried with oil-free compressed air. Enamel and dentin with phosphoric acid pre-etching (phosphoric acid applied for 15 seconds prior to application of the adhesive) or without phosphoric acid pre-etching (phosphoric acid was not applied) were also prepared.

Surface Free Energy Measurements

After preparation, the samples for surface free energy measurement were divided into two sets. One set of substrate samples was left untreated after the specimen preparation. The adhesives were applied to each of the various surfaces according to the manufacturers' instructions in the other set, and this set was light irradiated for 10 seconds with a quartz-tungsten halogen unit (Optilux 501, Kerr, Orange, USA). The power density (above 600 mW/cm^2) of the quartz-tungsten halogen unit was checked using a dental radiometer (model 100, Kerr) before preparing the specimens. Contact angles were measured to investigate the surface free energy characteristics of the various substrates and of the substrate treated by adhesive after light irradiation.

The surface free energy characteristics of the various substrates and of substrates treated by adhesive after light irradiation were determined by measuring the contact angles formed with the surface by three test liquids—bromonaphthalene, diiodomethane, and distilled water—each of which has known surface free energy parameters. For each test liquid, the equilibrium contact angle (θ) was measured by the sessile drop method under ambient conditions of 23°C ± 2°C and 50% ± 10% relative humidity using a contact angle measurement apparatus (DM 500, Kyowa Interface Science, Saitama, Japan) for 10 specimens per group. The apparatus was fitted with a charge-coupled device camera to enable automatic measurement. A standardized 1-μL drop of each test liquid was placed on the cured adhesive and uncured resin composite surfaces, and a profile image was captured after 500 ms using the apparatus. Contact angles were then calculated by the θ/2 method using the built-in interface measurement and analysis system (FAMAS, Kyowa Interface Science).

The surface free energy parameters of the solids were then calculated based on the fundamental concepts of wetting. The Young-Dupré equation describes the work of adhesion (W) between a solid (S) and a liquid (L) in contact as follows:

$$W_{SL} = \gamma_L + \gamma_S - \gamma_{SL} = \gamma_L(1 + \cos\theta).$$

Here, γ_{SL} is the interfacial free energy between the solid and liquid, γ_L is the SFE of the liquid, and γ_S is the surface free energy of the solid. By extending the Fowkes equation, as developed by Kitazawa-Hata, γ_{SL} can be expressed as follows:

$$\gamma_{SL} = \gamma_L + \gamma_S - 2(\gamma_L^d\gamma_S^d)^{1/2} - 2(\gamma_L^p\gamma_S^p)^{1/2} - 2(\gamma_L^h\gamma_S^h)^{1/2}$$

$$\gamma_{\mathrm{L}} = \gamma_L^e + \gamma_L^p + \gamma_L^h, \gamma_{\mathrm{S}} = \gamma_S^d + \gamma_S^p + \gamma_S^h,$$

where γ_L^d, γ_L^p, and γ_L^h are components of the surface free energy arising from the dispersion force, the polar force, and the hydrogen bonding force, respectively. Surface free energy values were determined for the three test liquids, and the surface free energy parameters were calculated based on these equations using the built-in software.

Shear Bond Strength (SBS) Test

The various substrates were prepared as described above. An Ultradent Bonding Assembly (Ultradent Products) was used for determining SBS. The adhesives were applied to the various substrates according to the manufacturers' instructions. Following the application of the adhesive to the bonding sites, bonded resin composite cylinders were formed on the adherends by clamping plastic molds (2.4 mm in internal diameter, approximately 2.5 mm in height) in the fixture against the various substrates. The resin composite (Clearfil AP-X, Kuraray Noritake Dental) was inserted all at once into the mold and then light irradiated for 40 seconds. The plastic mold was removed, and the finished specimens were transferred to distilled water and stored at 37°C for 24 hours, after which they were randomly allocated to two groups (n=25 per group) for thermal cycling: 1) no thermal cycling (24 h group); 2) 10,000 TC between 5°C and 55°C (TC group). Thermal cycling was conducted using a thermocycling machine (Thermal Shock Tester TTS-1 LM, Thomas Kagaku, Tokyo, Japan). Each cycle consisted of water-bath incubation for 30 seconds, with a transfer time of five seconds.

SBS measurements were performed using a universal testing machine (Type 5500R, Instron Worldwide Headquarters, Norwood, MA, USA) equipped with an Ultradent shearing fixture at a crosshead speed of 1.0 mm/min. An Ultradent shear bond test with a semicircular blade of 2.4-mm diameter was used for SBS measurement. The SBS values (MPa) were calculated from the peak load at failure divided by the bonding area. After testing,

Table 2: *Surface Free Energy and their Parameters of Various Substrates*[a]

Substrate	γ_S	γ_S^d	γ_S^p	γ_S^h
Enamel (etching)	71.6 (2.4) A	41.1 (0.7) A	11.3 (1.5) A	19.2 (2.2) A
Enamel (no etching)	55.9 (3.5) B	40.6 (0.7) A	3.8 (1.5) B	11.5 (2.4) B
Dentin (etching)	62.3 (3.4) C	40.8 (0.5) A	4.3 (1.3) B	17.1 (3.0) A
Dentin (no etching)	67.6 (3.4) D	41.0 (0.6) A	6.3 (1.2) C	20.3 (3.3) A
Resin composite	54.0 (2.4) B	40.4 (0.3) A	5.6 (1.2) C	8.0 (1.2) C
Lithium disilicate	69.0 (2.4) D	40.8 (0.6) A	9.1 (1.2) D	19.1 (3.4) A
Leucite glass ceramic	70.2 (2.1) AD	41.1 (0.6) A	9.6 (1.2) D	19.5 (2.4) A
Zirconia	67.9 (2.4) D	41.0 (0.6) A	9.0 (1.5) D	17.9 (2.3) A
Type III gold alloy	64.2 (2.1) C	40.9 (0.6) A	7.1 (1.0) C	16.2 (1.4) D
Au-Ag-Pd alloy	62.0 (2.9) C	40.7 (0.3) A	6.5 (1.2) C	14.9 (1.2) E

Abbreviation: Au-Ag-Pd alloy, gold-silver-palladium alloy.
[a] *Unit: mN/m; values in parenthesis are standard deviations (n=10). Same letter in vertical columns indicates no significant difference (p>0.05).*

the specimens were examined under an optical microscope (SZH-131, Olympus, Tokyo, Japan) at a magnification of 10× to assess the type of the bond failure. The proportions of the resin composite surface with adherent and visible remnants were estimated and used to classify the failure as follows: 1) adhesive failure; 2) cohesive failure in the substrate, 3) cohesive failure in the resin composite; and 4) mixed failure (combination of adhesive and cohesive failure).

Statistical Analysis

The surface free energy and SBS data obtained were analyzed using a commercial statistical software package (SPSS Statistics Base, IBM, Armonk, NY, USA). A one-way analysis of variance (ANOVA) and Tukey post hoc test were used for surface free energy data, and a two-way ANOVA and Tukey post hoc test were used for analysis of SBS data, with a significance level of 0.05.

RESULTS

Surface free energy Measurement of Various Substrates and Substrates Treated by Adhesive

The results for the surface free energy and their parameters of the various substrates are shown in Table 2. The surface free energy and their parameters of the various substrates show significant differences depending on the type of substrate used. The influence of the treatment with universal adhesives of the various substrates on surface free energy and their parameters is shown in Table 3. Surface free energy and their parameters of substrates treated by adhesive after light irradiation did not show any significant differences among the substrates, and the interfacial characteristics of substrates treated by adhesive after light irradiation were closer to those of untreated RC than those of various substrates.

SBS Tests of Universal Adhesives to Various Substrates

The influence of type of substrate on the SBS of universal adhesives 24 h and TC groups is shown in Figures 1 and 2. The two-way ANOVA revealed that the type of substrate and adhesive used did have a significant influence on SBS 24 h and TC groups. In addition, there was a significant effect for the interaction of the type of substrate and adhesive for SBS 24 h and TC groups. The failure modes of debonded specimens after SBS tests are shown in Table 4. Failure type was not associated with SBS, and the predominant type of failure seen was adhesive failure.

SBS of Universal Adhesives to Enamel

The SBSs of universal adhesives to enamel with and without phosphoric acid pre-etching 24 h and TC groups ranged from 25.7 ± 3.6 to 36.7 ± 4.4 MPa. The SBSs of universal adhesives to enamel with phosphoric acid pre-etching 24 h and TC groups were significantly higher than those without phosphoric acid pre-etching and did not depend on the type of adhesive used.

SBS of Universal Adhesives to Dentin

The SBSs of universal adhesives to dentin with and without phosphoric acid pre-etching 24 h and TC groups ranged from 26.6 ± 3.2 to 31.2 ± 4.2 MPa. The SBSs of universal adhesives to dentin with and without phosphoric acid pre-etching were similar and did not depend on the type of adhesive. In addition, the SBSs to dentin of TC group were higher

Table 3: *Influence of the Treatment with Universal Adhesives of the Various Substrates*[a]

Substrate	Adhesive	γ_S	γ_S^d	γ_S^p	γ_S^h
Enamel (etching)	SU	59.1 (2.2) A	40.6 (0.2) A	7.1 (0.6) A	11.4 (1.0) A
	GB	58.8 (1.9) A	40.3 (0.3) A	7.0 (0.5) A	11.5 (1.2) A
Enamel (no etching)	SU	57.8 (1.8) A	40.5 (0.8) A	6.1 (0.9) A	11.2 (1.9) A
	GB	57.0 (1.7) A	40.4 (0.7) A	5.8 (0.5) A	10.8 (1.8) A
Dentin (etching)	SU	58.8 (1.8) A	40.5 (0.7) A	6.8 (0.9) A	11.5 (1.8) A
	GB	58.3 (1.5) A	40.3 (0.4) A	6.4 (0.5) A	11.6 (1.1) A
Dentin (no etching)	SU	57.4 (1.8) A	40.5 (0.8) A	6.0 (0.9) A	10.9 (1.4) A
	GB	57.2 (1.8) A	40.4 (0.7) A	5.9 (0.5) A	10.9 (1.6) A
Resin composite	SU	59.1 (2.1) A	40.5 (0.3) A	7.0 (0.8) A	11.6 (1.1) A
	GB	58.6 (2.0) A	40.4 (0.3) A	7.0 (0.7) A	11.3 (1.2) A
Lithium disilicate	SU	57.6 (1.8) A	40.4 (0.8) A	6.1 (0.9) A	11.1 (1.2) A
	GB	57.3 (1.7) A	40.4 (0.6) A	6.1 (0.9) A	10.8 (1.2) A
Leucite glass ceramic	SU	57.9 (1.7) A	40.4 (0.8) A	6.2 (0.9) A	11.3 (1.8) A
	GB	57.2 (1.7) A	40.4 (0.7) A	6.0 (0.7) A	10.8 (1.6) A
Zirconia	SU	58.5 (1.8) A	40.5 (0.5) A	6.4 (0.9) A	11.6 (1.9) A
	GB	57.2 (1.3) A	40.3 (0.5) A	6.1 (0.7) A	10.8 (1.5) A
Type III gold alloy	SU	58.2 (1.8) A	40.5 (0.5) A	6.4 (0.9) A	11.3 (1.5) A
	GB	57.5 (1.8) A	40.3 (0.5) A	6.0 (0.7) A	11.2 (1.3) A
Au-Ag-Pd alloy	SU	58.3 (1.8) A	40.5 (0.4) A	6.3 (0.9) A	11.5 (1.5) A
	GB	57.4 (1.5) A	40.3 (0.4) A	6.1 (0.7) A	11.0 (1.4) A

Abbreviations: Au-Ag-Pd alloy, gold, silver, palladium alloy; GB, G-Premio Bond; SU, Scotchbond Universal.
[a] *Unit: mN/m; values in parenthesis are standard deviations (n==10). Same letter in vertical columns indicates no significant difference (p>0.05).*

than those of 24 h group, regardless of the presence or absence of phosphoric acid pre-etching.

SBS of Universal Adhesives to Resin Composite

The SBSs of universal adhesive to RC 24 h and TC groups ranged from 30.4 ± 3.9 to 34.5 ± 2.6 MPa and also did not show any significant differences depending on the type of adhesive used.

SBS of Universal Adhesives to Glass Ceramics

The SBSs of universal adhesives to LD and LR ranged from 2.9 ± 1.9 to 13.9 ± 4.1 MPa, and the SBSs of GP to LD and LR 24 h and TC groups were higher than those of SU.

SBS of Universal Adhesives to Zirconia

The SBSs of universal adhesives to ZR 24 h and TC groups ranged from 16.1 ± 3.1 to 28.8 ± 3.7 MPa. Although the SBSs of SU to ZR of 24 h group were significantly higher than those of GB, SU showed a significantly lower SBS to ZR of TC group than did GB.

SBS of Universal Adhesives to Metal Alloys

The SBS of universal adhesives to GA and AP ranged from 8.2 ± 3.0 to 18.8 ± 3.4 MPa. The SBSs to GA and AP of GB of 24 h and TC groups were significantly higher than those of SU.

DISCUSSION

The present study indicated that the surface free energy (γ_S,) and their parameters (γ_S^p and γ_S^h) of the various substrates was material dependent, but there were no significant differences in γ_S^d values between the types of substrates. It has been reported that the γ_S^d values of oxidized surfaces measured using this method are generally approximately 40 mN/m.[20,21] On the other hand, the γ_S^p value, which reflects polar interactions, and the γ_S^h value, which relates to the water and hydroxyl components, together measure hydrophilic interactions.[9] Therefore, it may be assumed that the interfacial characteristics of various substrates were influenced by the hydrophilicity of the tested surface, which depends on the different compositions of the various substrates.

The surface free energy and their parameters of substrates treated by adhesive after light irradiation did not show any significant differences among the substrates. After treatment with the current single-step adhesives, the substrates are covered with adhesive, which forms a thin layer (less than 10

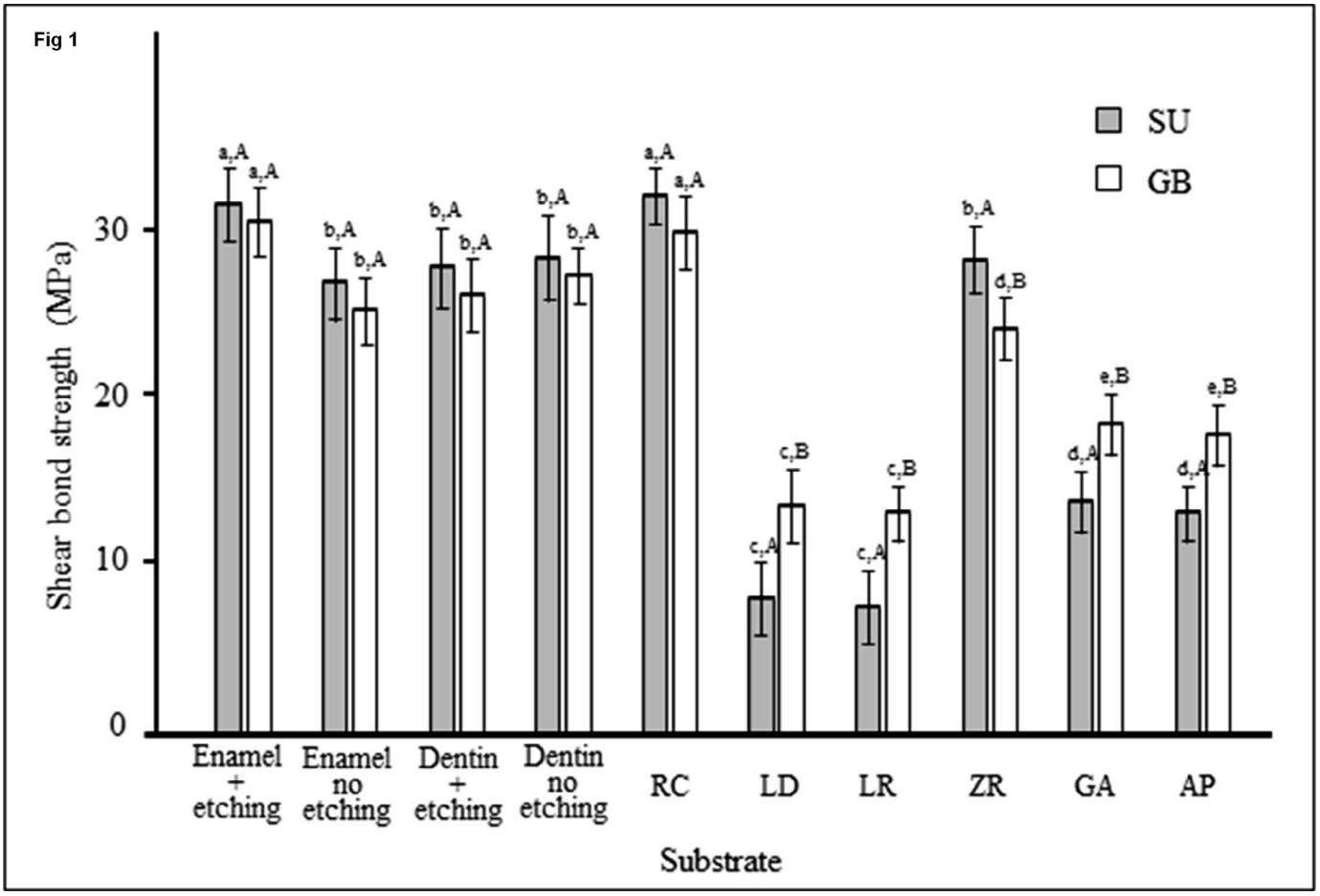

Figure 1. *Influence of type of substrate on the shear bond strength (MPa) of universal adhesives after 24 hours. The same lowercase letter indicates no significant differences between types of substrate. The same capital letter indicates no significant differences between adhesives. Abbreviations: SU, Scotchbond Universal; GB, G-Premio Bond; RC, resin composite; LD, lithium disilicate; LR, leucite reinforced; ZR, zirconia; GA, gold alloy, AP, gold-palladium.*

μm).[22] Although this raises a concern that the substrates might influence the surface properties or, as a result of incomplete wetting, might still be exposed in some locations, these results indicate that the universal adhesive achieves a consistent surface on a wide range of substrates.

The interfacial characteristics of substrates treated by adhesive after light irradiation were closer to those of untreated RC than those of various substrates. Optimal wettability is important to enable materials to spread across the entire surface and to establish adhesion.[23,24] Although the surface free energy of the adhesive surface must be maximized, the maximum bond strength is assumed to arise when the surface free energy parameters of the resin composites are close to those of the adhesive treated surface.[8] Therefore, the interfacial characteristics of substrate treated by adhesive after light irradiation are similar to those of resin composite, resulting in effective adhesion sites that have a proper balance between the surface free energy parameters of adhesive coated surfaces and resin composite. These results indicate that universal adhesives modify the interfacial characteristics of a wide range of substrates and coat them to create a consistent surface. Thus, in the clinic, it is possible to use these adhesives with a wide range of substrates, as long as the procedures are followed carefully. Overall, the results of this study require rejection of the null hypothesis that the interfacial characteristics of the various substrates would not be influenced by treatment with universal adhesives.

Although the modifying and coating ability of universal adhesives with substrates might improve their bond durability to various substrates, in the present study bond durability of universal adhesive to various substrates was different depending on the type of substrate and the adhesive. One of the key

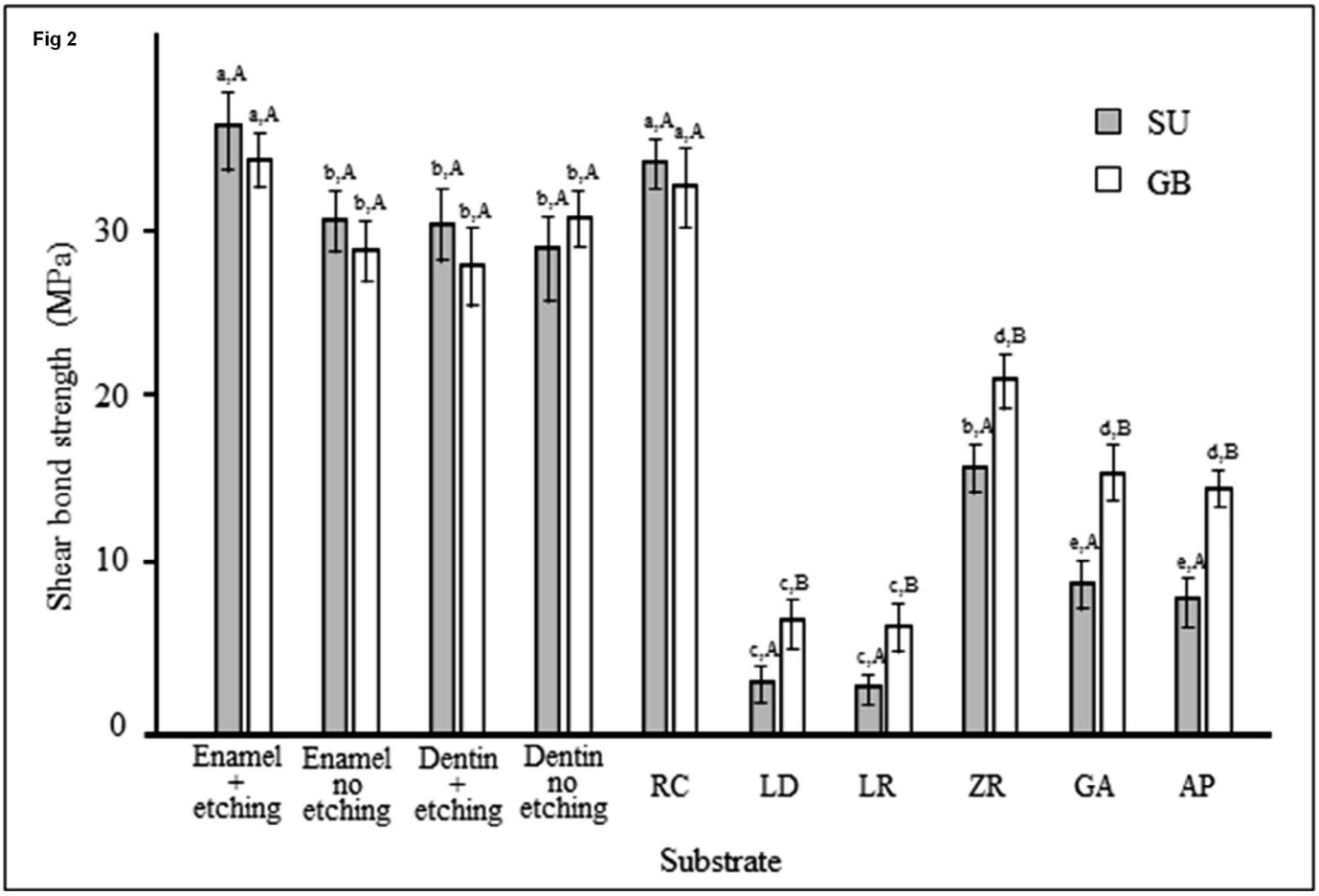

Figure 2. *Influence of type of substrate on the shear bond strength (MPa) of universal adhesives after 10,000 thermal cycles. The same lowercase letter indicates no significant differences between types of substrate. The same capital letter indicates no significant differences between adhesives. Abbreviations: SU, Scotchbond Universal; GB, G-Premio Bond; RC, resin composite; LD, lithium disilicate; LR, leucite reinforced; ZR, zirconia; GA, gold alloy, AP, gold-palladium.*

Table 4: *Failure Mode Analysis of Debonded Specimens after Shear Bond Strength Tests of Universal Adhesives with Various Substrates*[a]

Substrate	24 h group		TC group	
	SU	GB	SU	GB
Enamel (etching)	[23/2/0/0]	[23/2/0/0]	[20/3/1/1]	[20/4/1/0]
Enamel (no etching)	[23/2/0/0]	[20/4/1/0]	[23/0/1/1]	[23/0/1/1]
Dentin (etching)	[20/3/2/0]	[21/2/2/0]	[20/2/3/0]	[21/2/2/0]
Dentin (no etching)	[19/4/2/0]	[23/1/1/0]	[23/2/0/0]	[20/1/2/1]
Resin composite	[19/1/5/0]	[19/0/5/1]	[19/0/4/2]	[21/0/3/1]
Lithium disilicate	[25/0/0/0]	[25/0/0/0]	[25/0/0/0]	[25/0/0/0]
Leucite glass ceramic	[25/0/0/0]	[25/0/0/0]	[25/0/0/0]	[25/0/0/0]
Zirconia	[25/0/2/0]	[23/0/2/0]	[25/0/0/0]	[25/0/0/0]
Type III gold alloy	[25/0/0/0]	[30/0/0/0]	[25/0/0/0]	[25/0/0/0]
Au-Ag-Pd alloy	[25/0/0/0]	[25/0/0/0]	[25/0/0/0]	[25/0/0/0]

Abbreviations: Au-Ag-Pd alloy, gold, silver, palladium alloy; GB, G-Premio Bond; SU, Scotchbond Universal; TCs, thermal cycles.
[a] *[ ] Indicates failure mode [adhesive failure/cohesive failure in substrate/cohesive failure in resin/mixed failure].*

factors for success with universal adhesives is the chemical bonding capability of their functional monomers to various substrates.[3-7] Therefore, the chemical bonding potential between the adhesive and various substrates may have a greater influence on the bond durability of universal adhesives than do their surface modification and coating effects.

The SBSs of universal adhesives to enamel with phosphoric acid pre-etching of 24 h and TC groups were significantly higher than those without phosphoric acid pre-etching and did not depend on the type of adhesive. Over the years, phosphoric acid pre-etching has become the standard procedure for enamel conditioning to improve surface characteristics prior to the application of adhesive bonding agents.[25] Phosphoric acid pre-etching of enamel increases not only the bonding area but also the wettability of the adherent surface.[26] In addition, the surface free energy of enamel with phosphoric acid pre-etching was significantly higher than that of enamel without phosphoric acid pre-etching in the present study. The evidence from several studies,[3,27,28] including the current study, clearly shows an increase of enamel bond strength following phosphoric acid pre-etching.

The SBSs of universal adhesives to dentin with and without phosphoric acid pre-etching of each of the 24 h and TC groups were similar and did not depend on the type of adhesive. Previous studies[3,29] have demonstrated that the MDP in universal adhesive allows for stable bonding to dentin regardless of the presence or absence of phosphoric acid pre-etching. This monomer forms a stable nanolayer together with a deposition of stable MDP–calcium salts at the adhesive interface regardless of the presence or absence of phosphoric acid pre-etching, which increases the bond strength of the adhesive interface.[30] SU also contains a specific polyalkenoic acid copolymer (Vitrebond copolymer) used in the resin-modified glass ionomer Vitrebond (3M ESPE).[31] Vitrebond copolymer bonds chemically and spontaneously to hydroxyapatite, and a previous study[32] demonstrated a higher bond strength for an adhesive containing it than for an adhesive without the Vitrebond copolymer. On the other hand, GB also contains 4–methacryloxyethyl trimellitic acid (4-MET) as a functional monomer. It has been reported[33] that 4-MET has a strong chemical bonding potential to calcium-containing substrates, similar to MDP. Therefore, chemical interactions between hydroxyapatite and specific components of the adhesive can be thought to lead to the higher SBS of universal adhesives to dentin regardless of the presence or absence of phosphoric acid pre-etching.

The SBSs to enamel and dentin of TC group were higher than those of 24 h group, regardless of the presence or absence of phosphoric acid pre-etching. The mechanical properties of the adhesive interface might improve over time as a result of post-curing within the adhesive and the resin composites, resulting in SBS to enamel and dentin of TC group that is higher than that of 24 h group.

The SBS of the universal adhesives to RC of 24 h and TC groups did not show any significant differences depending on the type of adhesive used. For composite-composite bonding, a previous study[34] has suggested that the use of an intermediary layer is beneficial to improve surface wetting and chemical bonding, regardless of the texture created by the mechanical surface treatment. The use of an intermediary layer purportedly enhances composite-composite bonding by promoting chemical coupling to the resin matrix, chemical bonds to the exposed filler particles, and micromechanical retention through monomer penetration into the microstructure of the resin composite.[35] This may be why universal adhesive creates a strong bond between cured resin composite and newly applied resin composite.

The SBSs of universal adhesives to LD and LR of TC group were significantly decreased compared to those of 24 h group. The use of silane coupling agents in enhancing the bond of resin composite to silica-based ceramics is widely accepted[36] and thus is used with universal adhesives for bonding to ceramics.[6,37] Silane is a dual functional monomer consisting of a silanol group that reacts with the ceramic surface and a methacrylate group that co-polymerizes with the adhesives.[38] However, it has been reported[36] that a rapid increase in the amount of water absorbed by the adhesive interface causes hydrolysis and degradation of the silane. Water storage and thermal cycling have been described[39] as detrimental for silane-ceramic bonding. In addition, it has been reported[40] that silanized interfaces appear to be unstable in humid conditions, and the silane bond was found to deteriorate in moisture. Since the current adhesives are permeable to water, the silane-ceramic bond is expected to deteriorate by hydrolysis over time. Therefore, it appears that the SBS of universal adhesive to LD and LR of TC group is influenced by the detrimental effects on silane-ceramic bonding from the thermal cycling. SU contains silane and MDP monomer, which aids in the adhesion of resin to ceramics, and thus this adhesive is capable of bonding with ceramics without

the addition of any silanating step.[6] This kind of universal adhesive provides a new, simplified approach to bonding between resins and ceramics.[37] However, the results of the present study show that GB, which requires an additional silanating step prior to applying the universal adhesive, has a higher SBS of 24 h and TC groups than does SU. Therefore, this result suggests that when applying a universal adhesive, an additional silanating step may be valuable for optimizing the ceramic-resin bond even with universal adhesives.

The present study showed that the SBS of universal adhesives to ZR of 24 h and TC groups was significantly higher than that of LD and LR, and the universal adhesives bonded well to zirconia. MDP has also been shown[41] to be effective in improving resin bonding to zirconia. It has been assumed that the hydroxyl groups of the phosphate moiety in MDP interact with the hydroxyl groups on the zirconia surface through Van der Waals forces or hydrogen bonds.[5] Therefore, the results for SBS to ZR for universal adhesives may be explained by the chemical bonding affinity between MDP and zirconia. In addition, the SBS of SU of 24 h group was significantly higher than that of GB. Silane cannot contribute to the chemical bond to zirconia because zirconia lacks silica. However, silane could increase the wettability of the zirconia surface and as a result improve the initial bond strength.[42] However, in spite of the high SBS of 24 h group, SU showed a significantly lower SBS to zirconia than did GB of TC group. The silane may increase the hydrophilicity of SU, thereby predisposing the adhesive layer to hydrolytic degradation. This hypothesis needs further investigation.

The SBSs to GA and AP of GC of 24 h and TC groups were significantly higher than those of SU. Generally, metal elements are classified into two categories: noble metals (e.g., gold, palladium, or silver) and base metals (e.g., copper and aluminum).[43] For bonding to noble metals, methacrylate monomers that contain sulfur have been synthesized and used clinically.[44] A methacryloxyalkyl thiophosphate methylmethacrylate, a sulfur-containing monomer, is used in GB to improve bonding with noble metals. In contrast to noble metals, base metals are characterized by an oxide layer, which is created on the metal surface in an atmospheric environment. Although MDP chemically bonds to oxidized base metals, the base metal content of GA and AP typically only ranges from 15% to 20%. These results suggest that universal adhesives employing sulfur-containing monomers together with a phosphate monomer may be effective for bonding with dental metals. According to the results of this study, the other null hypothesis, that there would not be differences in bond durability of universal adhesive to various substrates, can also be rejected.

The results of this study suggest that universal adhesives modify the interfacial characteristics of a wide range of substrates and create a consistent surface, but that the bond durability of universal adhesive to various substrates differs depending on the type of substrate and the adhesive. Therefore, it is crucial for general practitioners who use universal adhesives to understand the factors that contribute to the bond durability with various substrates and to be aware of the chemical bonding potential between adhesives and substrates.

CONCLUSIONS

The results of this study suggest that the interfacial characteristics of the untreated surfaces of the various substrates show significant differences depending on the type of substrate, but that the interfacial characterics of substrates treated by universal adhesive after light irradiation do not show any significant differences regardless of the substrate used. The interfacial characteristics of substrates treated by universal adhesive after light irradiation were closer to those of resin composite than those of various substrates. This modifying and coating ability of universal adhesives is expected to contribute to the bond durability of universal adhesive with various substrates, especially in an adhesive-resin composite interface. However, the bond durability of universal adhesives to various substrates differs depending on the type of substrate and the adhesive used and is particularly variable with glass ceramics, zirconia, and metal alloys. This indicates that the difference in bond durability was strongly influenced by the chemical bonding potential between substrates and universal adhesives.

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Regulatory Statement

This study was conducted in accordance with all of the provisions of the local human subjects oversight committee guidelines and policies of the Ethics Committee for Human and Animal Studies at Nihon University School of Dentistry in Tokyo, Japan. The approval code for this study is 2014-10.

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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Microleakage and Shear Bond Strength of Composite Restorations Under Cycling Conditions

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Clinical Relevance

The performance of composite restorations can be affected by frequent acid attacks.

SUMMARY

Objectives: The aim of this study was to evaluate microleakage and shear bond strength of composite restorations under different cycling conditions.

Methods and Materials: Class V cavities were prepared in the buccal and lingual surfaces of 30 human molars (n=60). A further 60 molars were used to prepare flat enamel and dentin specimens (n=60 each). Cavities and specimens were divided into six groups and pretreated with an adhesive (self-etch/Clearfil SE Bond or etch-and-rinse/Optibond FL). Composite was inserted in the cavities or adhered to the specimens' surfaces, respectively, and submitted to cycling (control: no cycling; thermal cycling: 10,000 cycles, 5°C to 55°C; thermal/erosive cycling: thermal cycling plus storage in hydrochloric acid pH 2.1, 5 minutes, 6×/day, 8 days). Microleakage was quantified by stereomicroscopy in enamel and dentin margins after immersion in silver nitrate. Specimens were submitted to shear bond strength testing. Statistical analysis was done by two-way analysis of variance and Kruskal-Wallis tests ($p<0.05$).

Results: Microleakage in enamel margins was significantly lower in the control group compared with thermal cycling or thermal/erosive cycling. Erosive conditions increased microleakage compared with thermal cycling (significant only for Clearfil SE Bond). No significant differences were observed in dentin margins. Bond strength of enamel specimens was reduced by thermal cycling and

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thermal/erosive cycling when Clearfil SE Bond was used and only by thermal/erosive cycling when Optibond FL was used. No differences were observed among dentin specimens.

Conclusions: Thermal/erosive cycling can adversely affect microleakage and shear bond strength of composite resin bonded to enamel.

INTRODUCTION

Erosive tooth wear often requires restorative treatment due to hypersensitivity or esthetic or functional limitations when a certain degree of substance loss is reached. As restorative materials and adhesive techniques have been significantly optimized over the past decades, the restoration of even severely eroded teeth using minimally invasive procedures has become possible. While minimal loss of tooth substance can be protected from further progression by sealant application,[1-3] distinct defects can be restored by composite, resin polymer, or ceramic restorations after minimal or even no preparation.[4-8]

Some researchers have purported that adhesion to eroded dental hard tissues might be more difficult to achieve than adhesion to sound enamel and dentin. While only few studies showed that erosion of tooth surfaces does not jeopardize enamel[9] or dentin[10] bonding, most experiments have found a reduced bond strength of adhesives to eroded dental hard tissues.[11-16] However, both adhesion to erosively affected dental hard tissues and the performance of dental restorations under ongoing erosive conditions are of clinical relevance, in cases where a causal therapy of erosive tooth wear cannot be achieved before restoration placement. While several studies have investigated the acid-resistance of different restorative materials per se,[17-22] only a few studies have analyzed the effect of erosive attacks on dental restorations.[23,24] These studies reported some surface erosion of enamel adjacent to cement and composite restorations but did not analyze potential effects on the adhesive interface, for example on microleakage or bond strength of composite restorations.[23,24] Studies on secondary caries development have shown that the application of self-etching adhesives with specific functional monomers, for example 10-methacryloyloxydecyl dihydrogenphosphate (MDP), leads to the formation of a so-called acid-base resistant zone beneath the dentinal hybrid layer[25] or at the enamel-bonding interface.[26] This zone is more resistant to acid and base challenges than the underlying dental hard tissue and might prevent caries development at the tooth-restoration interface. However, the acid challenge of the interface was usually performed with buffer solutions at pH 4.5[25-27] and no information is currently available on the acid resistance of the interface when the demineralizing agent is significantly more acidic, as with erosive solutions.

Therefore, this study aimed to investigate the effect of erosive cycling on the adhesive performance of an etch-and-rinse and a self-etch adhesive by investigating microleakage and shear bond strength of composite restorations. The null hypothesis tested was that erosive challenges do not influence microleakage in enamel and dentin margins of Class V restorations and do not affect shear bond strength of self-etch and etch-and-rinse adhesive systems to enamel and dentin.

METHODS AND MATERIALS

Cavity and Specimen Preparation

Ninety sound human third molars were collected after approval of the local ethics committee (No: 1.190.857), cleaned, and stored in distilled water under refrigeration for less than 3 months.

Thirty teeth were selected for microleakage analysis, and Class V cavities (4 mm in diameter and 1.5 mm in depth) were prepared in the cervical region of the buccal and lingual surfaces using wheel-shaped diamond burs (#909 ISO040, Maxima, Gillingham, United Kingdom) in an air/water cooled high-speed handpiece. Each bur was replaced after five preparations. The cavity margins were located in enamel and dentin as the gingival cavosurface margins were placed 2 mm below the cementoenamal junction. The enamel surface was beveled 0.5 mm using a flame-shaped diamond bur (#832.014 EF, Komet, Gebr. Brasseler, Lemgo, Germany). The cavity size (4 mm $\times$ 1.5 mm) was checked with a periodontal probe. The teeth were randomly assigned into six groups (n=5 teeth, each with two cavities, one for each adhesive used).

For shear bond strength analysis, the roots of the remaining 60 teeth were removed and the crowns cut in a mesial-distal direction. The specimens were embedded in acrylic resin (Paladur, Heraeus Kulzer, Hanau, Germany), and subsequently, the buccal or lingual surfaces were flattened with water-cooled silicon carbide discs (#600 grit, Water Proof Silicon Carbide Paper, Struers, Ballerup, Denmark) until an area with diameter of at least 3 mm in enamel (n=60) or dentin (n=60) was exposed. The specimens were randomly assigned to six test groups (each n=10 enamel and n=10 dentin specimens) according to the adhesive (etch-and-rinse or self-etch adhesive)

Table 1: *Products, Manufacturers, Batch Numbers, Chemical Compositions and Application Instructions for the Materials Tested*

Material	Composition	Application Protocol
Clearfil SE Bond 2 Self-etch (Kuraray, Okayama, Japan)	*Primer (batch: 2B0133)*: 10-MDP, HEMA, hydrophilic dimethacrylate, N,N-diethanol-p-toluidine, di-camphorquinone, water.	Primer applied over tooth substrate actively for 20 s, followed by gentle air-dry for solvent evaporation. Then, application of a thin layer of Bond and light curing for 10 s.
	Bond (batch: 2901214): 10-MDP, BIS-GMA, HEMA, hydrophobic dimethacrylate, N,N-diethanol-p-toluidine, di-camphorquinone, silanated colloidal silica	
Optibond FL Etch & Rinse (Kerr, Orange, CA, USA)	*Primer (batch: 5086326)*: HEMA, PAMM, GPDM, ethanol, water, photoinitiator	Phosphoric acid etching for 30 s in enamel and 15 s in dentin, followed by rinsing with water spray for 30 s. Primer applied over tooth substrate actively for 15 s, followed by gentle air-dry for 5 s to solvent evaporation. Then, application of a thin layer of bond and light-curing for 20 s.
	Bond (batch: 5417219): TEG-DMA, UDMA, GPDM, HEMA, BIS-GMA, filler, photoinitiator	
Filtek Supreme XTE (3M ESPE, St Paul, MN, USA)	*Monomer:* UDMA, BIS-GMA TEG-DMA, BIS-EMA	Application in increments of 2 mm, each followed by light curing for 20 s.
	Filler: 58% volume/volume aggregated zirconia/silica cluster filler and nonagglomerated/nonagregated silica filler. (batch: N669171)	

Abbreviations: MDP, 10-methacryloyloxydecyl dihydrogenphosphate; HEMA, 2-hydroxyethyl methacrylate; BIS-GMA, bisphenol A glycidyl methacrylate; PAMM, phthalic acid monoethyl methacrylate; GPDM, glycerylphosphate dimethacrylate; TEG-DMA, triethylene glycol dimethacrylate. UDMA, urethane dimethacrylate. BIS-EMA, bisphenol A ethoxylate dimethacrylate.

and cycling conditions (no aging/control, thermal cycling or thermal/erosive cycling) used.

Material Application and Aging Conditions

The cavities (for microleakage analysis) or specimens (for shear bond strength analysis), respectively, were treated with an etch-and-rinse adhesive (Optibond FL, Kerr, Orange, CA, USA) or a self-etch adhesive (Clearfil SE Bond 2, Kuraray, Okayama, Japan). For the etch-and-rinse technique, 35% phosphoric acid (Ultra-Etch, Ultradent Inc, South Jordan, UT, USA) was applied onto enamel and then extended to dentin, resulting in 30 seconds of enamel etching and 15 seconds of dentin etching. The adhesives were applied according to the manufacturer's recommendations (Table 1) and light cured (800 mW/cm^2, Optima 10, B.A. International, Northampton, United Kingdom).

Class V cavities were restored with a composite (Filtek Supreme XTE, shade A2, 3M ESPE, St Paul, MN, USA; Table 1) in increments of 2 mm, each light-cured for 20 seconds. After 24 hours, the restorations were finished with a sequence of polishing disks (Sof Lex Pop-On, 3M ESPE) in decreasing roughness.

For shear bond strength analysis, composite was adhered on the enamel or dentin surfaces. After adhesive application, a Teflon split mold (3 mm in diameter, 2 mm in height) was used on the surface, the composite packed against the surface and then light-cured for 20 seconds. After light-curing, the Teflon mold was split and removed.

In the control group, microleakage and shear bond strength analyses were performed after 24 hours of water storage in distilled water. The remaining specimens were submitted to thermal cycling (10,000 cycles, 5/55° C, with a dwell time of 25 seconds and a transfer time of 5 seconds) or thermal/erosive cycling (thermal cycling plus intermittent immersion in hydrochloric acid at room temperature, pH 2.1, six times a day for 5 minutes with an interval of 90 minutes between the acid immersions). The thermal cycling regimen lasted for 8 days, so that the erosive cycling lasted a total of 4 hours.

Microleakage Analysis

The root apices of the teeth were sealed with composite resin (Filtek Supreme XTE) and then coated with two layers of nail polish leaving an area of 1 mm around the margin interface uncoated. The teeth were immersed in 25% volume/volume $AgNO_3$ for 12 hours, followed by immersion in a photo-developing solution (GBX Developer and Replenisher, Carestream Dental, Atlanta, GA, USA) for 8 hours under ultraviolet light.

The teeth were rinsed under running water for 5 minutes and cut in four parallel slices of 1-mm thickness in a buccal-lingual direction, parallel to the tooth long axis. Slices were analyzed under the stereomicroscope with 25× magnification (Carl Zeiss

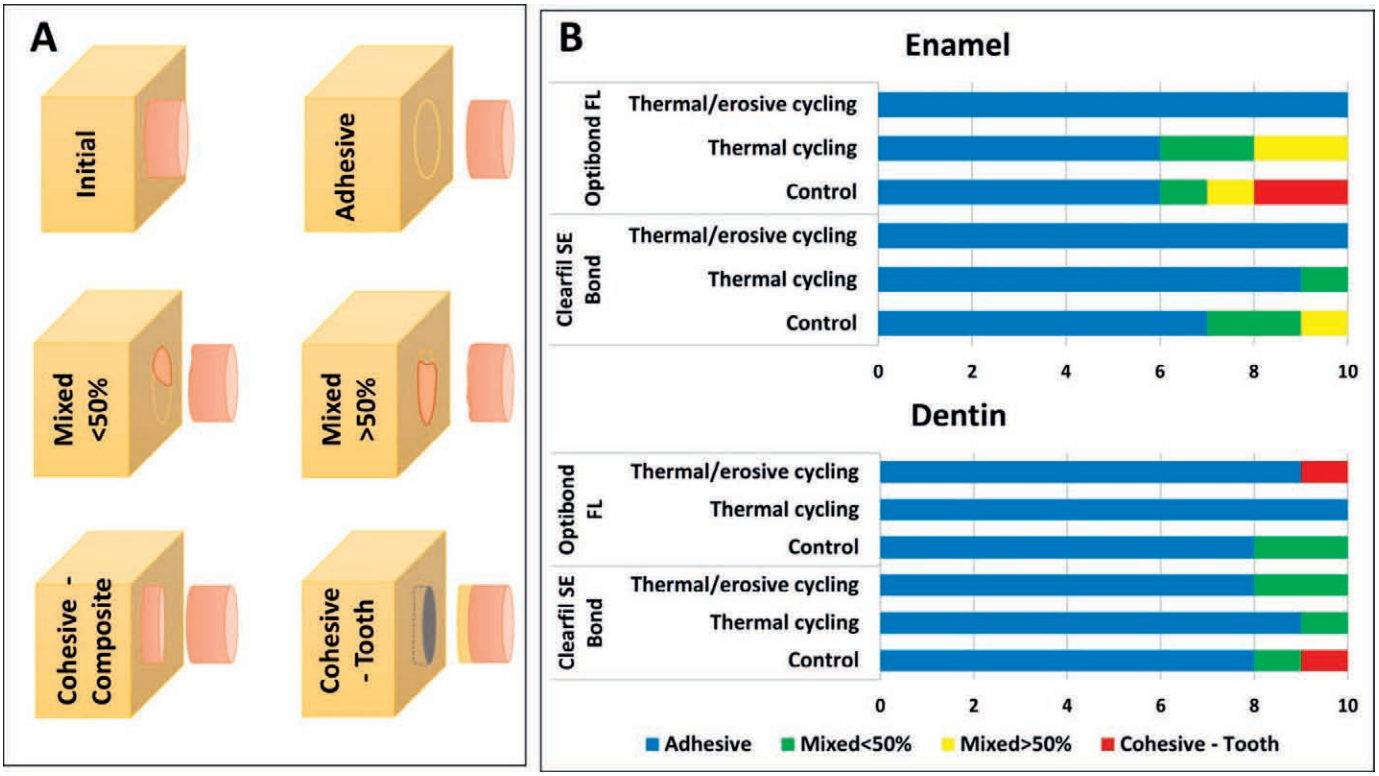

Figure 1. *Failure pattern type and distribution.(A) Schematic drawing of the failure patterns. Adhesive: failure in the adhesive interface; mixed <50%: failure in the composite, with <50% of its total area; mixed >50%: failure in the composite, with >50% of its area; cohesive-tooth: failure affecting the substrate (enamel/dentin) and cohesive-composite: failure affecting only the composite. (B) Failure distribution of enamel and dentin specimens. No cohesive failures were observed in composite.*

Inc, Berlin, Germany), and images were taken by a digital camera (Moticam 2.0, Motic, Hong Kong). The penetration of silver nitrate was measured using the software AxioVision LE 2013 (Carl Zeiss Inc). Data were expressed in percentage of penetration using the formula: %p = [P / L] * 100, where *%p* is the percentage of penetration, *P* is the length of the margin where the silver penetrated and *L* is the total length of enamel/composite or dentin/composite interface.

Additionally, one specimen from each group was randomly chosen and used for scanning electron microscopy (SEM) of the surface margins. Before immersion in the $AgNO_3$ solution, impressions were made using polyvinylsiloxane (President Light Body, Coltene, Altstätten, Switzerland), followed by a preparation of the epoxy replicas (EpoFix, Struers, Ballerup, Denmark). The epoxy resin was placed under vacuum for 20 minutes and cured in a desiccator for 24 hours to avoid the formation of bubbles. Replicas were sputtered with palladium-platinum, and SEM analysis (ULTRA Plus FE-SEM, Carl Zeiss Inc, Oberkochen, Germany) was performed at 200× magnification.

Shear Bond Strength Analysis

Shear bond strength was tested with a universal testing machine (Z010, Zwick GmbH & Co, Ulm, Germany). A shear force was applied to the enamel-composite or dentin-composite interface, respectively, through a chisel-shaped loading device positioned parallel to the enamel or dentin surface at a crosshead speed of 1 mm/min. Shear bond strength (σ) was calculated using the load at failure F (N) and the adhesive area A (mm^2): σ (MPa) = F/A. The debonded area was examined with a stereomicroscope (Carl Zeiss Inc.) at 25× magnification, and the failure modes were classified into one of four categories: adhesive, if it occurred in the adhesive interface; mixed <50%, if occurred in the composite with <50% of the total area adhered to the tooth substrate; mixed >50% if occurred in the composite with >50% of the total area adhered to the tooth substrate; and as cohesive-tooth if the failure affected only the dental substrate (enamel or dentin) or cohesive-composite if the failure affected only the composite. Figure 1A shows a schematic drawing of the failure modes.

Table 2: *Microleakage (Percent of Silver Penetration, Means and Standard Deviations) of Enamel and Dentin Margins* [a]

	Enamel		Dentin	
	Clearfil SE Bond	**Optibond FL**	**Clearfil SE Bond**	**Optibond FL**
Control	10.3 ± 13.4^{Aa}	8.9 ± 11.0^{Aa}	56.0 ± 33.5^{Aa}	61.5 ± 34.4^{Aa}
Thermal cycling	45.6 ± 21.8^{Ab}	31.3 ± 13.3^{Ab}	65.8 ± 30.2^{Aa}	68.0 ± 24.4^{Aa}
Thermal/erosive cycling	71.5 ± 12.6^{Ac}	41.2 ± 19.5^{Bb}	88.6 ± 11.5^{Aa}	77.2 ± 20.5^{Aa}

[a] *Separately for enamel and dentin, different uppercase letters show differences between the adhesives, while lowercase letters show significant differences between the aging conditions.*

Statistical Analysis

Means and standard deviations were determined for each subgroup. Normal distribution was tested by the Kolmogorov-Smirnov test.

Microleakage data were normally distributed for enamel but not for dentin, so two-way analysis of variance (ANOVA) followed by Tukey tests were applied to the enamel data, while the Kruskal-Wallis test was used to analyze the dentin data. As shear bond strength data were normally distributed, two-way ANOVAs followed by Tukey tests were applied separately for enamel and dentin. Considering the kind of tooth substrate and the kind of adhesive used, a χ^2 test was applied to compare the failure patterns in the different aging subgroups. The level of significance was set at 5%.

RESULTS

Microleakage

Microleakage of Class V restorations is presented in Table 2. For enamel, the type of adhesive ($p<0.0001$), the cycling condition ($p<0.0001$), and the interaction between factors ($p=0.004$) were significant with respect to microleakage. Microleakage was significantly lower in the control group than in restorations that were submitted to thermal cycling or thermal/erosive cycling. Thermal/erosive cycling increased microleakage compared with thermal cycling, but this effect was significant only for Clearfil SE Bond. In dentin, thermal cycling and thermal/erosive cycling increased microleakage slightly but not significantly.

Examples of surface margins of each group are shown in Figures 2 through 4. While continuous

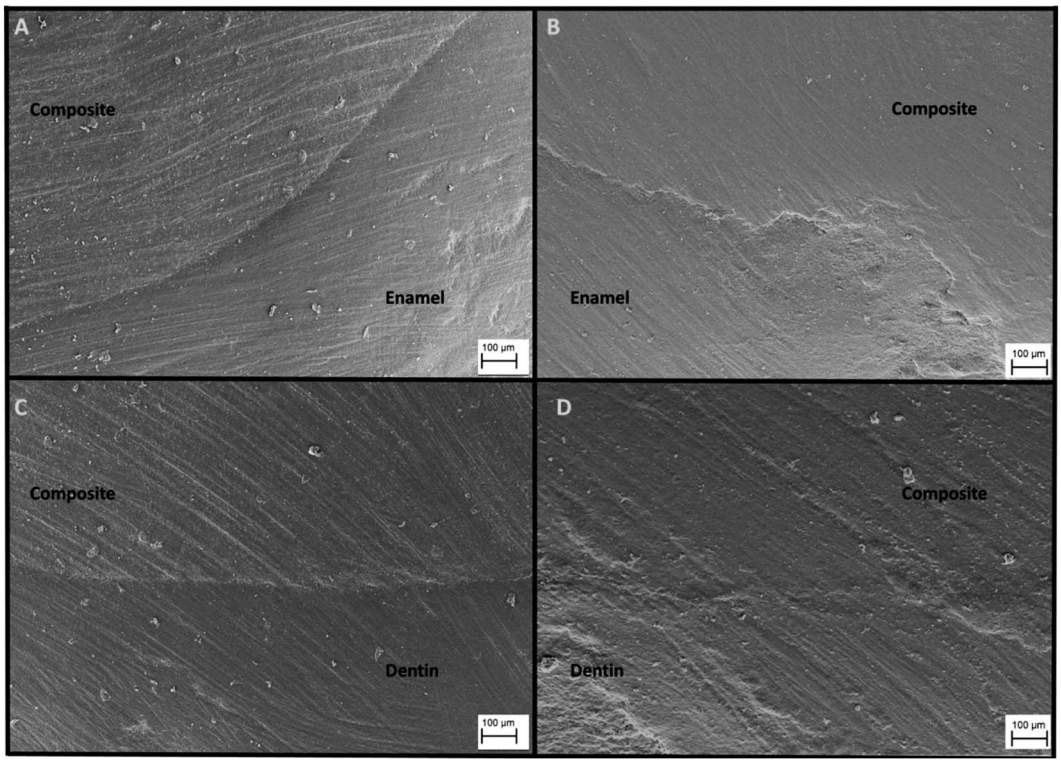

Figure 2. *SEM figures (×200) showing composite-enamel (A, B) and composite-dentin (C, D) interfaces in the control groups when Clearfil SE Bond (A, C) or Optibond FL (B, D) was used. In all restorations, continuous margins were found.*

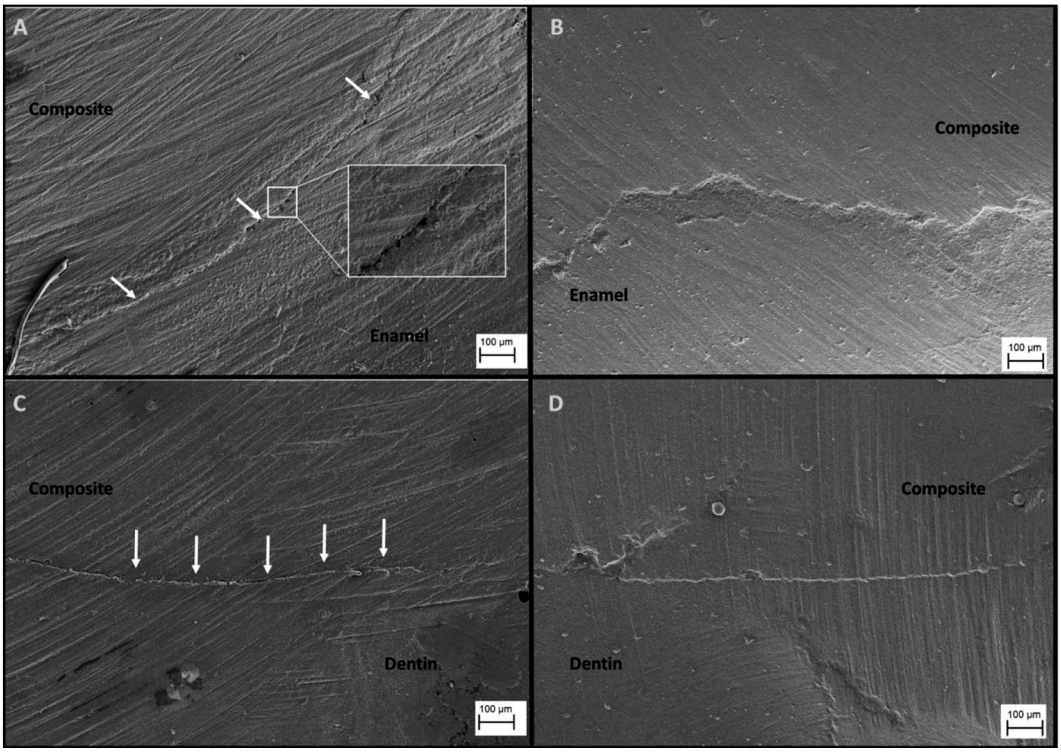

Figure 3. *SEM figures (×200) showing composite-enamel (A, B) and composite-dentin (C, D) interfaces in restorations submitted to thermal cycling when Clearfil SE Bond (A, C) or Optibond FL (B, D) was used. A slight disintegration is visible for Clearfil SE Bond (arrows) compared with the Optibond FL sample. The box in (A) presents the composite-enamel interface at 2800× magnification.*

margins were found in the control groups (Figure 2), thermal cycling resulted in a slight disintegration of the restoration performed with Clearfil SE Bond (Figure 3). Thermal/erosive cycling resulted in a distinct dissolution of enamel and dentin margins (Figure 4).

Shear Bond Strength

Shear bond strength values of differently aged enamel and dentin specimens are presented in Table 3.

For enamel, two-way ANOVA revealed significant effects of cycling treatment ($p<0.0001$) and adhesive type ($p=0.0003$) but not for the interaction between the factors ($p=0.641$). When Clearfil SE Bond was used, thermal cycling and thermal/erosive cycling reduced bond strength significantly compared with the control, but they were not significantly different from each other. When Optibond FL was used, only thermal/erosive cycling reduced shear bond strength significantly compared with the control, while thermal cycling led to a nonsignificant reduction of bond strength.

For dentin, two-way ANOVA revealed no effects of cycling conditions ($p=0.994$) or type of adhesive ($p=0.709$) on shear bond strength, while the interaction of both factors was significant ($p=0.043$).

The failure distribution is presented in Figure 1. Independently of the kind of substrate and the kind of adhesive, χ^2 tests revealed no significantly different failure patterns among the different cycling conditions ($p=0.15$).

DISCUSSION

As thermal/erosive cycling adversely affected microleakage and shear bond strength of enamel but not dentin, the null hypothesis had to be partially rejected.

In the present study, two basic techniques to determine the effect of thermal/erosive cycling on the adhesive interface were used. Microleakage and shear bond strength analyses are surrogate parameters, which are critically discussed in the literature,[28,29] but are still frequently used. As relative effects of thermal/erosive cycling on the composite-tooth interface rather than absolute bond strength or microleakage values were of interest, it seemed acceptable to apply both methods.

Optibond FL as an etch-and-rinse adhesive and Clearfil SE Bond as a self-etching adhesive have

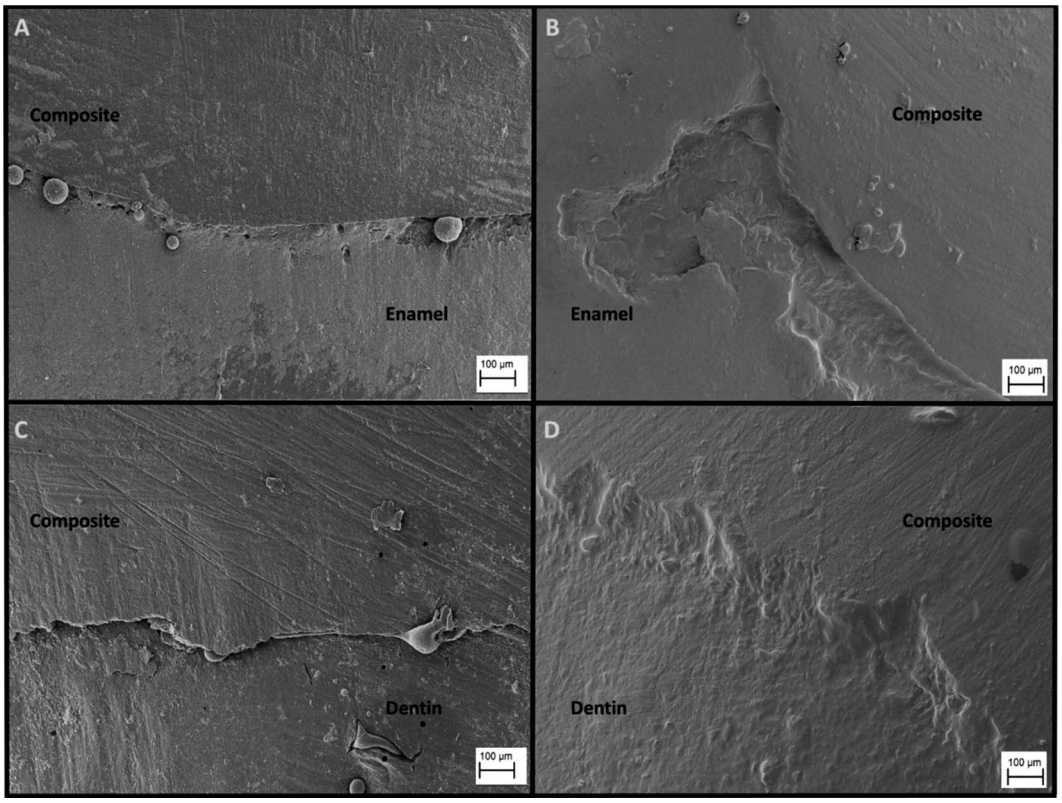

Figure 4. *SEM figures (×200) showing composite-enamel (A, B) and composite-dentin (C, D) interfaces submitted to erosive cycling when Clearfil SE Bond (A, C) or Optibond FL (B, D) was used. Erosive cycling resulted in a distinct surface dissolution of enamel or dentin, respectively, resulting in a step between composite and the tooth surface.*

shown reliable adhesive performance and are considered benchmarks in their respective classes; they are known to be quite resistant to aging.[30] Cycling of enamel specimens resulted in a significant decrease of bond strength and microleakage, while shear bond strength and microleakage of dentin specimens were not significantly changed. Previous studies on the effect of artificial aging found conflicting results. Depending on the kind of aging protocol, some studies showed a significant decrease in bond strength for Optibond FL or Clearfil SE Bond on enamel or dentin surfaces after aging,[31-34] while others did not.[35-37] Microleakage of composite restorations bonded by Optibond FL or Clearfil SE Bond mostly increased by aging.[38,39] Different *in vitro* models to promote the degradation of the adhesive interface have been described in the literature, including aging by storage in water or NaOCl, enzymatic degradation of the organic matrix, thermocycling, pH cycling, or mechanical loading.[40] In the present study, specimens were submitted to 10,000 cycles in water between 5°C and 55°C, which corresponds approximately to 1 year of *in vivo* service.[41] Thermal cycling might accelerate hydrolysis compared with aging by water storage and induce repetitive contraction-expansion stress at the tooth/restoration interface[40]. Specimens submitted to thermal/erosive cycling were intermittently stored in hydrochloric acid at pH 2.1 in addition to thermal cycling. Hydrochloric acid is commonly used to

Table 3: *Shear Bond Strength (MPa, Means and Standard Deviations) of Enamel and Dentin Specimens* [a]

	Enamel		Dentin	
	Clearfil SE Bond	**Optibond FL**	**Clearfil SE Bond**	**Optibond FL**
Control	16.2 ± 5.1^{Aa}	20.0 ± 5.3^{Aa}	14.6 ± 5.5^{Aa}	12.8 ± 5.6^{Aa}
Thermal cycling	8.7 ± 3.5^{Ab}	14.9 ± 4.8^{Bab}	11.0 ± 3.8^{Aa}	16.3 ± 5.4^{Aa}
Thermal/erosive cycling	6.8 ± 2.4^{Ab}	10.6 ± 5.4^{Ab}	14.8 ± 5.2^{Aa}	12.8 ± 5.1^{Aa}

[a] *Separately for enamel and dentin, different uppercase letters show differences between the adhesives, while lowercase letters show significant differences between the aging conditions.*

simulate intrinsic erosion.[42,43] However, the erosive cycling can be classified as relatively mild when considering that the intraoral pH after an acidic attack is reduced for up to several minutes[44] and the total erosion time in the present study lasted for only 4 hours.

As depicted by the SEM pictures, a distinct surface erosion of enamel and dentin developed at the marginal interface. The adverse effect of hydrochloric acid on the marginal interface might increase the flow of fluids through the adhesive interface accounting for microleakage development and decrease of bond strength. However, microleakage and shear bond strength were significantly affected only in enamel but not in dentin.

Bonding to enamel surfaces is mainly achieved by a micromechanical interlocking of resin into microporosities of the acid-etched surface. In contrast, the dentinal hybrid layer is composed of organic matrix, residual hydroxyapatite crystallites, and resin monomers. As erosion primarily affects the inorganic part of the dental hard tissue, the effects on enamel bonding might be more deleterious than on dentin. Nevertheless, it has taken into account that the degradation of the organic matrix was not addressed by the aging protocol of the present study. Considering that intrinsic erosion is caused by gastric juices, not only hydrochloric acid but also proteolytic enzymes of the digestive system (eg, pepsin) come into contact with teeth. Pepsin is capable of degrading the organic matrix of dentin, resulting in a progression of erosive lesions.[45]

In this experiment, only slight differences were found between the adhesive performance of the etch-and-rinse and the self-etching adhesive. Etch-and-rinse adhesives usually lead to higher bond strength values and lower microleakage on enamel than self-etching adhesives, as phosphoric acid etching increases the porosity of enamel compared with the mild etching pattern of the Clearfil SE Bond primer, resulting in an increased micromechanical retention. Thus, Optibond FL revealed higher bond strength values and less adhesive failures after thermal cycling compared with Clearfil SE Bond. On the other hand, the mildly acidic primer of Clearfil SE Bond can easily decalcify the less mineralized dentin. At the same time, MDP can chemically interact with hydroxyapatite resulting in improved dentin bonding performance compared with etch-and-rinse adhesives. In contrast to etch-and-rinse adhesives, self-etching adhesives with specific functional monomers form an acid-base resistant zone, a structural layer on the tooth-bonding interface, which might be responsible for degradation resistance at the interface. Nevertheless, no significant differences between the performance of the etch-and-rinse and the self-etching adhesive under thermal/erosive cycling conditions were seen in the present study. Further studies should analyze possible structural changes of the hybrid layer under highly acidic conditions.

Conclusion

Erosive conditions might adversely affect microleakage development and bond strength of etch-and-rinse and self-etching adhesives on enamel but not on dentin.

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 ICT-UNESP. The approval code for this study is 1.190.857.

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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Biological Effects of Provisional Resin Materials on Human Dental Pulp Stem Cells

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Clinical Relevance

Possible cytotoxicity from chemical-activated provisional resin materials should be considered to avoid pulp tissue injury, particularly in extensively prepared teeth.

SUMMARY

Objectives: This study investigated the *in vitro* cytotoxicity as well as the proinflammatory cytokine expression of provisional resin materials on primary cultured human dental pulp stem cells (hDPSCs).

Methods: Five commercially available provisional resin materials were chosen (SNAP [SN], Luxatemp [LT], Jet [JE], Revotek LC [RL], and Vipi block [VB]). Eluates that were either polymerizing or already set were added to hDPSCs under serially diluted conditions divided into three different setting times (25% set, 50% set, and 100% set) and incubated for 24 hours with 2× concentrated culture media. Cell cytotoxicity tests were performed by LDH assay and live and dead confocal microscope images. The expression of proinflammatory cytokines in SN and VB was measured using cytokine antibody arrays. Data were analyzed using repeated measures analysis of variance (ANOVA) or ANOVA followed by the Tukey *post hoc* test at a significance level of $p<0.05$.

Results: Cytotoxicity greater than 30% was observed in the 50% diluted culture in SN, LT, and JE in the already set stage ($p<0.05$), while it was detected in SN and LT in early or intermediate stage samples. The cytotoxicity of SN, JE, and LT was greater with eluates from the polymerizing phase compared to that from already set samples ($p<0.05$), as observed by live and dead images. On the other hand, RL and VB did not exhibit cytotoxicity greater than 30%. Proinflammatory cytokines were not detected in 12.5% diluted culture with eluates from VB and early set stage SN.

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Conclusions: The eluates from chemical-activated provisional resin materials during polymerization (SN, LT, and JE) were cytotoxic to hDPSCs and may adversely affect pulp tissue.

INTRODUCTION

Provisional resin materials are widely used for interim restorations in operative dentistry to maintain the marginal integrity of prepared teeth, protect the pulp tissue, ensure occlusal function, and provide an acceptable esthetic appearance until the adjustment of final restorations.[1] They present easy handling, suitable mechanical properties, and low cost.[2,3]

Provisional resin materials are classified into five categories based on how the restoration is obtained during processing: 1) chemical-activated, 2) heat-activated, 3) light-activated, 4) dual-activated, and 5) computer-aided design and computer-aided manufacture (CAD/CAM) acrylic resins.[4,5] There are several commercially available provisional resin materials with various types of monomers: 1) polymethyl methacrylate (PMMA), 2) polyethyl methacrylate (PEMA), 3) urethane dimethacrylate (UDMA), 4) other types or combinations of unfilled methacrylate resins, and 5) composite resins of bisphenol A–glycidyl methacrylate (Bis-GMA) or Bis-acryl.[5-7]

For initial biocompatibility testing of dental materials, cytotoxicity tests with various types of cells derived from the oral tissue have been investigated and adverse cellular effects are considered to be the result of released toxic components.[8-13] Many researchers have used oral fibroblasts and keratinocytes to investigate the cytotoxicity of provisional resin materials on oral mucosa.[5,14,15] However, there have been no reports concerning their cytotoxicity with regard to dental pulp cells even though material eluants may diffuse into pulp tissue via open dentinal tubules. When the exposed dentin of prepared teeth is exposed to provisional resin materials, toxic material components can reach the pulp tissue via open dentinal tubules and damage these tissues, which are more highly sensitive to such components than is oral mucosa.[16-20] Even worse, provisional resin materials undergoing polymerization accelerate adverse effects to pulp tissue. When being polymerized, provisional resin materials are able to release more unreacted monomers or toxic eluates when placed on prepared teeth.[21-23]

In addition to cytotoxicity, dental pulp stem cells are adversely affected by chemicals to produce numerous proinflammatory cytokines/chemokines, including interleukin-6 (IL-6), IL-8, chemokine (C-C motif) ligand 2 (CCL2), and C-X-C motif ligand 12 (CXCL12), which increase the likelihood of irreversible pulp inflammation. Chemokines, such as CCL2 and CXCL12, are a family of small cytokines and act as chemoattractants to guide the migration of immune cells and induce an inflammatory reaction.[24-26] Therefore, proinflammatory cytokines/chemokines from dental pulp stem cells are investigated after stimulation by eluates from provisional resin materials.[5,14]

In this experiment, we performed cytotoxicity tests to determine the effects on primary cultured human dental pulp stem cells (hDPSCs) observed during the early or intermediate stage of polymerization with the provisional resin materials compared to the already set stage counterpart. In addition, the proinflammatory cytokine expression of eluates from provisional resin materials was tested to reveal the possible adverse response. The null hypothesis was that the cytotoxic effects on hDPSCs of extracts from the polymerizing materials and those from the already set materials would not differ. The second null hypothesis was that proinflammatory cytokine expression from provisional resin materials did not show any difference compared to controls under less cytotoxic concentrations.

METHODS AND MATERIALS

Provisional Resin Materials

Five provisional resin materials were used in this study: chemical-activated polyethyl methacrylate resin (SN; Snap, Parkell Inc, Edgewood, NY, USA), chemical-activated bis-acryl composite resin (LT; Luxatemp, DMG, Englewood, NJ, USA), chemical-activated polymethyl methacrylate resin (JE; Jet, Lang Dental, Wheeling, IL, USA), light-activated urethane dimethacrylate resin (RL; Revotec LC, GC America, Alsip, IL, USA), and CAD/CAM fabricated polymethyl methacrylate resin blocks (VB; Vipi block, Madespa, Río Jarama, Toledo, Spain). Table 1 shows their general chemical compositions.

Preparation of Specimens

Each provisional resin material was placed within a Teflon mold 10 mm in diameter and 2 mm in height. When the mixed material reached the early "dough" stage, it was packed into a mold and covered with a cover glass. The polyethyl methacrylate resins (SN) and polymethyl methacrylate resins (JE) were measured, hand mixed, and autopolymerized. The powder was weighed using an electronic balance

Table 1: *Provisional Resin Materials Tested in This Study*

Product	Code	Manufacturer	Lot Number	Composition
Snap	SN	Parkell Inc	01707	Polyethyl methacrylate (PEMA)
Jet	JE	Lang Dental	40142	Polymethyl methacrylate (PMMA)
Luxatemp	LT	DMG	720535	Bis-acrylic composites
Revotec LC	RL	GC America Inc	1409191	Urethane dimethacrylate (UDMA)
Vipi block	VB	Madespa	0000034924	Polymethyl methacrylate (PMMA)

(Explorer, Ohaus Corporation, Parsippany, NJ, USA), and the liquid was measured by volume. For each material, the manufacturer-recommended powder-to-liquid ratio was used. For LT, the material was mixed using the dispensing syringe. The material was dispensed into the mold and allowed to autopolymerize. RL was hand molded and placed into the Teflon molds, and LED light (Litex 695, Dentamerica, City of Industry, CA, USA) was applied for polymerization. Each group was divided into three different setting times (25% set, 50% set, and 100% set) and was immediately placed in distilled water (DW). RL was placed in the Teflon mold and the specimen was either unpolymerized or polymerized by a light-curing unit (Litex 695) for 10 or 20 seconds. It was immediately placed in DW for extraction. The PMMA block (VB) was CAD/CAM processed according to the manufacturer's protocol and was sterilized using ultraviolet light for 1 hour after the application of ethylene oxide gas. The specimen was placed into DW. The experimental conditions are summarized in Table 2.

Collection of Resin Extract

Each provisional resin specimen was extracted at a ratio of 3 cm^2/mL following the recommendations of ISO 10993-12.[27] Because the surface of the sample was 2.2 cm^2, they were incubated in 0.73 mL of medium. For each provisional resin material except VB, nine specimens were fabricated and divided into three groups for a different extraction starting point. Three specimens were used for extracting VB. To simulate the clinical environment, eluates of all specimens were collected for 24 hours at 37°C in a shaking incubator (120 rpm). All procedures were performed on a sterilized clean bench to prevent any possible contamination. Extract was freshly gathered independently for each set of cytotoxicity and microarray tests.

Culture of hDPSCs

The hDPSCs were used at a low passage (below 10) throughout the experiments. After the experimental protocol was approved by the Institutional Review Board, the hDPSCs were gathered from extracted third molars from human adults. The cells were cultured in α-MEM supplemented with 10% fetal bovine serum (Gibco, Waltham, MA, USA), 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA), 2 mM of GlutaMAX (Gibco), and 0.1 mM of L-ascorbic acid (Sigma, St. Louis, MO, USA) in a humidified atmosphere at 37°C with 5% CO_2. All culture systems adhered to the above conditions.

hDPSCs Potency Analysis by Flow Cytometry

The hDPSCs grown at confluence in a six-well culture plate were detached with Accutase (Invitrogen), washed twice with phosphate-buffered saline (PBS; Welgene, Daegu, Korea), and then resuspended to 1×10^5 cells/mL. The harvested cells were fixed with 4% paraformaldehyde for 10 minutes and washed with PBS. Then each sample was treated with 0.2% Triton X-100 (Sigma) for 5 minutes and blocked using 1% bovine serum albumin (Sigma), and the cells were probed with primary antibodies, such as goat anti-CD105 and anti-CD73 antibodies and rabbit anti-CD 106, CD-34, and CD-45 antibod-

Table 2: *Experimental Conditions of Provisional Resin Materials*

Materials	Extraction Starting Time After Start of Mixing			Polymerizing Method
	Early Stage	Intermediate Stage	Already Set Stage	
Snap	2 min, 30 s (SN1)	5 min (SN2)	10 min (SN3)	Chemical
Luxatemp	1 min, 30 s (LT1)	3 min (LT2)	6 min (LT3)	Chemical
Jet	2 min, 30 s (JE1)	5 min (JE2)	10 min (JE3)	Chemical
Revotek LC	Unpolymerized (RL1)	10 s (RL2)	20 s (RL3)	Light
Vipi block	—	—	VB	Prepolymerized

ies at 1:100 dilution overnight at 4°C. The cells were then incubated with fluorescein isothiocyanate (FITC)-labeled goat secondary anti-goat or anti-rabbit antibodies at 1:100 dilution for 2 hours at room temperature in the dark, after which they were washed with PBS three times and kept on ice until analysis. All primary antibodies were obtained from Santa Cruz Ltd (Dallas, TX, USA). All FITC-labeled secondary antibodies were obtained from Invitrogen. Data were acquired using a sterile clean capped fluorescence-activated cell sorting (FACS) tube (5 ml) (BD Bioscience, San Jose, CA, USA) with 10,000 cells using FACS (Calibur Flow Cytometer, BD Bioscience) after all channels were calibrated by BD FACS caliber beads (BD Bioscience). Data were analyzed using Cell Quest-Pro software (BD Biosciences).

Cytotoxicity Tests and Cell Viability

Cytotoxicity tests were performed according to ISO 10093-5.[28] All specimens from each provisional resin group were cultured in a 96-well plate (SPL Life Sciences, Pocheon-si, Gyeonggi-do, Korea) at 1×10^4 cells/well with 100 μL of supplemented media for 24 hours at 37°C. After being washed with PBS, the cells were cocultured for another 24 hours with 2× supplemented media (50 μL), and serially diluted extract with DW (50 μL) was added. The percentages of the final concentrations of extracts in the culture media were 50%, 25%, 12.5%, 6.25%, and 3.125%. Samples in which only DW was added to hDPSCs served as the control group.

The potential cytotoxic effects on hDPSCs were evaluated by the quantity of extracellularly released cytosolic lactate dehydrogenase (LDH) using the CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI, USA). After the cell cultures were exposed to the material extracts for 24 hours, the cell culture supernatants were collected. The cell supernatants were centrifuged at 250*g* for 4 minutes to remove cell debris, and 50 μL of supernatants were transferred into an optically clear 96-well plate, followed by the addition of 50 μL of reagent. After incubation for 30 minutes at room temperature, a stop solution of 50 μL was added, and absorbance at 490 nm was measured using a microplate reader (SpectraMax M2e, Molecular Devices, Sunnyvale, CA, USA). The cytotoxicity results are expressed as percentage of extracellularly released LDH activity, calculated against the total (intracellular + extracellular) LDH activity, which was obtained after cell lysis buffer was added to hDPSCs. This percentage corresponds to the relative quantity of dead cells among the total cells in culture.

To evaluate live and dead cells, confocal images were obtained on a Zeiss LSM700 laser scanning microscope (Carl Zeiss, Thornwood, NY, USA). Cell viability was assessed with calcein AM (0.5 μM) and an ethidium homodimer-1 (4 μM) (Molecular Probes, Eugene, OR, USA) solution for 30 minutes, and they were examined under a confocal laser microscope (LSM700, Carl Zeiss). Green fluorescence was observed from the live cells and red fluorescence from the dead cells.

Cytokine Analysis

The 12.5% eluates from SN1 (early stage 1) and VN (already set stage 1), which showed less than 20% cytotoxicity, were selected for cytokine analysis because the 25% or 50% eluates may have cytotoxicity-induced cytokines. DW and 4 μg/mL of lipopolysaccharide (from *Escherichia coli*; LPS, Sigma) in DW were added to the same quantity of 2× supplemented media for negative and positive controls, respectively, to synchronize the total DW volume in total media. The Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA, USA) was used to determine the total protein concentrations for each of the conditioned media from the eluates. The same amount of total protein of the conditioned media was used for cytokine/growth factor analysis. A Proteome Profiler human cytokine array detection kit (Proteome Profiler, R&D Systems, Minneapolis, MN, USA) was used to detect cytokine/growth factor expression from the primary human dental pulp cells after exposure to the eluates from provisional resin specimens, as described by the manufacturer. Briefly, each membrane was blocked for 1 hour, incubated with the conditioned media at 4°C for 12 hours, washed, incubated with horseradish peroxidase–conjugated streptavidin for 30 minutes at room temperature, and finally washed before adding the detection agents for 1 minute according to the manufacturer's instructions. The signal from each membrane was examined with a chemiluminescence imager (ImageQuant LAS 4000, GE Healthcare, Little Chalfont, UK). The cytokine array test was repeated three times, and each cytokine was present twice on each membrane. The density of the protein spots was measured using a densitometry program (ImageQuant TL, Ver. 7.0., GE Healthcare). The positive control spots on the membranes were used to normalize the intensity

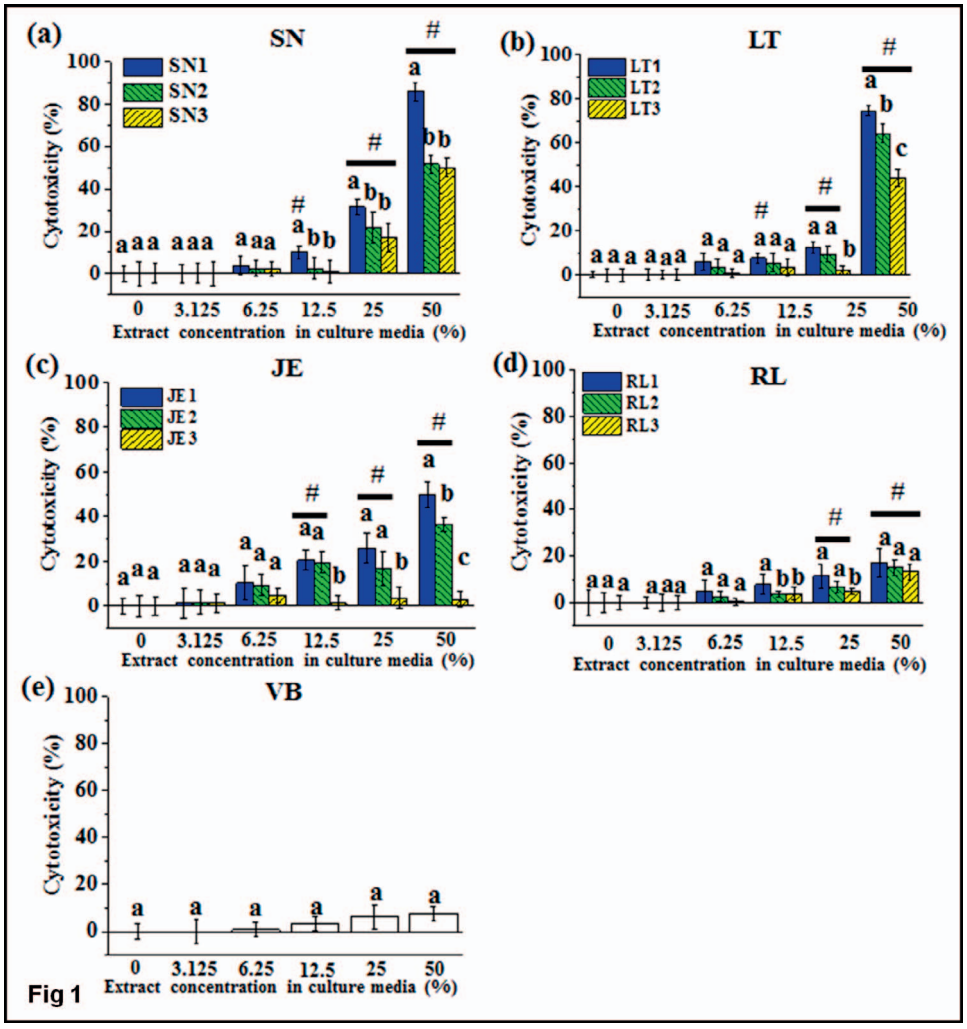

Figure 1. *Cell cytotoxicity results of five provisional resins according to the extract conditions (early, intermediate, and already set stage) and the concentration of extract in culture media with hDPSCs (0%, 3.125%, 6.25%, 12.5%, 25%, or 50%). Different letters indicate significant differences among the same extract concentration (p<0.05). # indicates a significant difference in cytotoxicity compared to 0% (p<0.05). Representative means ± standard deviations (n=6) are shown after experiments independently run in triplicate.*

between the different membranes to allow for comparison of the signal intensities.

Statistical Analysis

The cytotoxicity data from different extraction starting points were statistically analyzed by repeated measures analysis of variance (ANOVA). ANOVA was used for cytotoxicity comparison among serially diluted extract groups (50%, 25%, 12.5%, 6.25%, 3.125%, and 0%) within the same product and extract starting point. The Tukey *post hoc* test was used at levels of significance of $p<0.05$. The SPSS PASW version 23.0 software program (SPSS Inc) was used.

RESULTS

Stem Cell Plasticity of hDPSCs

Positive expression of CD73, CD105, and CD106 was detected in over 90% of hDPSCs, while they showed negative expression of CD34 and CD45 (not shown).

Cytotoxicity

The cytotoxicity test results for the LDH assay are shown in Figure 1. Extracellularly released LDH from hDPSCs of various provisional resins with three different states of polymerization was measured.

An early polymerizing state, intermediate polymerizing state, and final polymerized state were used to investigate the cytotoxicity to hDPSCs. Cell viability was determined by the leakage of LDH, an intracellular enzyme, from extract-conditioned groups compared to that from the total lysis group, which represented 0% cell viability. According to ISO 10993-5, 30% of cytotoxicity compared to the control was indicated as the cytotoxic threshold.[28]

Overall, after incubation with 50% extract, all extracts from SN, LT, JE, and RL showed significant differences in cytotoxicity compared to the DW-treated 0% control (Figure 1a-d, $p<0.05$). In con-

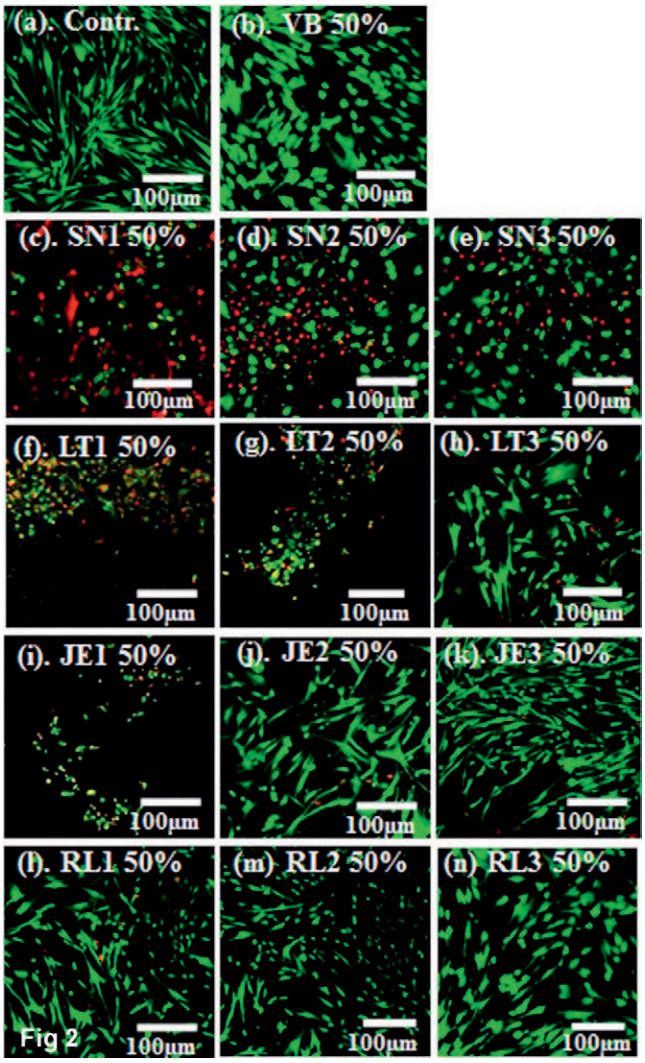

Figure 2. *Live (green) and dead (red) cells in 50% extract. Live and dead cells in media supplemented with 50% distilled water (DW) are shown as a control. Representative images are shown after experiments independently run in triplicate.*

trast, cell cytotoxicity was not significantly observed in the VB (Figure 1e, $p>0.05$), which was considered a negative control. All extract conditions from SN, JE, and LT except JE3 showed greater than 30% cytotoxicity, while cytotoxicity was significantly higher in the early or intermediate state than in the already set state for SN, JE, and LT (Figure 1a-c, $p<0.05$). RL showed relatively low cytotoxicity (10%-20%, Figure 1d).

After incubation with 25% extract, all extract conditions from SN, LT, JE, and RL, except LT3, JE3, and RL3, showed significant differences in cytotoxicity compared to the DW-treated 0% control (Figure 1a-c, $p<0.05$). Cell cytotoxicity over 30% was shown only in SN1, and there was a significant difference between SN1 (eluates from the early set stage) and SN3 (eluates from the already set stage) (Figure 1a, $p<0.05$). After incubation with 12.5%, 6.25%, and 3.125% extract, only 12.5% SN1, LT1, JE1, and JE2 exhibited significant cytotoxicity compared to 0% (Figure 1a-c, $p<0.05$), while cytotoxicity over 30% was not observed in any of the groups.

Severe cytotoxicity from 50% cultured conditions was reevaluated by confocal microscopic images after live and dead staining. The results are shown in Figure 2, in which live cells appear green and dead cells appear red. A significant number of dead cells but few live cells appeared in the early set, intermediate set, and already set stages of SN, JE, LT, and RL, whereas similar numbers of viable cells appeared in VB compared to the control. The early set and intermediate set stage showed dead cells with few live cells compared to the already set state in SN, JE, LT, and RL (Figure 2).

Proinflammatory Cytokines

Cytokine expression in conditioned media from extract-treated hDPSCs was compared to DW-treated hDPSCs in Figure 3. There was no significant difference in cytokine/chemokine expression in 12.5% SN1 and VB extract-treated hDPSCs compared to their DW-treated counterparts ($p>0.05$, Figure 3e), which showed MIF and SerpinE1expression. Additionally, the cytokine/chemokine expression of IL-8, CCL2, and CXCL12/SDF-1 was significantly observed in the LPS-treated positive group ($p<0.05$, Figure 3d,e).

DISCUSSION

Based on these results, the first null hypothesis that there would be no differences in the levels of cytotoxicity between materials that were in the process of polymerizing and those that had already set was rejected. However, we accepted the second null hypothesis that there would be no differences in the levels of proinflammatory cytokines from provisional resin materials compared to controls under less cytotoxic conditions.

In vitro cytotoxicity testing has been widely used to evaluate the biocompatibility of dental materials because it facilitates the reproducibility of testing results and provides highly reliable data from standardized protocols in a fast, inexpensive way.[28] *In vitro* cytotoxicity testing can be divided into two methods, direct and indirect, depending on the method of contact with mammalian cells. Direct

contact exposes a dental material or an extract from a dental material directly to mammalian cells, whereas indirect contact involves a barrier, such as agar, an artificial membrane filter, or dentin.

In this experiment, direct *in vitro* cytotoxicity tests were performed using extracts from five provisional resin materials and hDPSCs to mimic the clinical circumstances in which extracts from provisional resin materials could adversely affect pulp tissue via open dentinal tubules. The indirect cytotoxicity test using natural dentin (from human or bovine teeth) or artificial dentin (ie, Millipore filter) is considered to successfully reflect the intervention of dentin when pulp tissue is subjected to extracts from provisional resin materials. In this study, direct *in vitro* cytotoxicity tests were chosen to avoid natural dentin– and artificial dentin–dependent bias, which cause a lack of accessibility or of similarity to natural dentin structure, respectively. Gathering intact human dentin from adult tooth is possible only when third molar or premolar teeth are intentionally extracted for therapeutic reasons. In addition, established artificial dentin does not exist even though many attempts have been made to mimic the natural dentin structure and its biological/mechanical function.[29] Direct *in vitro* cytotoxicity testing using extracts also has many drawbacks, such as the inability to fully reproduce dentinal fluid and dentin structure. However, in this study, direct *in vitro* cytotoxicity tests using extracts provided an initial investigation for showing differences of cytotoxicity based on the extract starting time because of the advantage of the test's reproducibility. Further study using natural human dentin disks with artificial chambers is needed to confirm the extract starting time–dependent cytotoxicity for achieving clinical relevance.

In the development of cytotoxicity tests for dental materials, many attempts were made to mimic the clinical conditions of application. For example, the cytotoxicity of elastomeric impression materials was evaluated using human gingival fibroblasts with extract from the polymerizing and already set states, which revealed differences in cytotoxicity.[30] Other dental materials, such as zinc oxide eugenol, root canal sealers, resin adhesives, and resin-reinforced glass ionomer cements, have also been evaluated during and after setting, and all results showed an increase in cytotoxicity in the setting state compared to the already set state.[31-34] These cytotoxicity tests are considered to successfully mimic the clinical changes of polymerizing/setting dental materials which accelerate putative leaching or the entry of

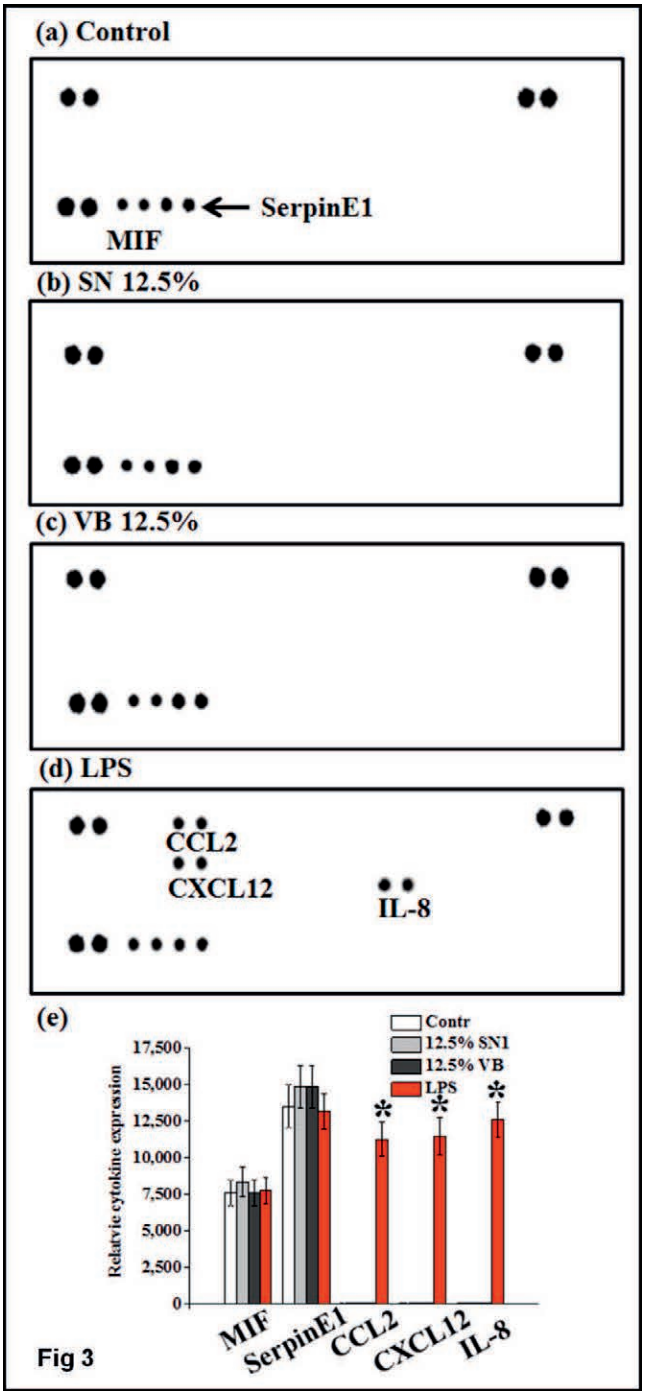

Figure 3. *Proinflammatory cytokine/chemokine expression in conditioned media from eluate-treated hDPSCs. After hDPSCs were treated with distilled water (DW) (a), 12.5% of SN1 (b), 12.5% of VN extract (c), or 4 μg/mL of LPS (d) for 24 hours, cytokine array experiments were performed. After normalizing the cytokine intensity from experimental groups to that from the DW-treated control group, the relative cytokine expressions are shown as means ± standard deviations (e). Means ± standard deviations are shown of experiments independently run in triplicate. Asterisks indicate significant differences compared to controls ($p<0.05$).*

dissolved substances resulting in contact with oral tissues.

Provisional resin materials can affect pulp tissue via open dentinal tubules when they are applied onto exposed dentin during polymerization. After the provisional materials have set, the eluates from provisional resins and temporary cementation material continuously make contact with dentin and possibly affect pulp tissue. Therefore, to mimic the application of provisional resin on prepared teeth with exposed dentin and to evaluate the possible adverse effects on the pulp tissue during the fabrication of provisional resin prostheses, extracts from provisional resin materials during and after polymerization were cultured with hDPSCs, which make up dental pulp tissue and determine the pulp's regenerative potential.

In vitro cytotoxicity testing using single cells with extract has many drawbacks, such as less clinical relevance compared to indirect human dentin barrier tests and an inability to be used in the tissue complex.[35] However, as an initial screening test, *in vitro* testing using affected tissue-specific cell lineage with extract from material is necessary to investigate the cytotoxicity of materials before conducting further clinically relevant *in vitro*, *in vivo*, or clinical studies, which sometimes take more time, cost more money, or raise ethical problems.[28] In this study, three different extraction starting times (early, intermediate, and already set stages) after the start of mixing were used for assessing clinical relevance in the polymerizing process. Within the limitation of the *in vitro* cytotoxicity test, results showed that polymerizing provisional resin materials (especially chemical-activated products) showed the potential to adversely affect dental pulp cells. To confirm these findings and to achieve more clinical relevance, *in vitro* cytotoxicity testing using human dentin samples with simulated dentinal fluid flow, *in vivo* animal tests, and clinical testing is recommended for further investigation.[36] From the above studies, the adverse effects from provisional materials on pulp tissue can finally be characterized in detail. Of course, it is also important to note that *in vivo* animal testing has the limitation of having unreliable accuracy in assessing pulpal compatibility for humans due to anatomical and biological differences.[37]

The hDPSCs are the main source of cells for maintaining the homeostasis of pulp tissue and accelerating the regeneration of pulp tissue.[38] Their natural function in the production of odontoblasts to create reparative dentin supports the regeneration of dentin structures by the production of the dentin/pulp-like complex as a protective barrier to the pulp tissue.[39] In addition, hDPSCs play a role in the immune response of pulp tissue against pulp damage by cytokine/chemokine production.[40,41] After confirming the stem cell plasticity of hDPSCs with positivity for mesenchymal stem cell markers (CD73, CD105, and CD106), which represent the potency to differentiate into osteogenic, chondrogenic, and adipogenic lineage,[42] and negativity for the hematological markers CD34 (early hematopoietic stem cell marker) and CD45 (hematolymphoid cell marker),[42-44] *in vitro* cytotoxicity studies were performed.

LDH is a natural cytosolic enzyme present in mammalian cells. When intact plasma membrane is damaged by the external environment, intracellular LDH is released into the cell culture media, which is recalled as extracellular LDH, an indicator of cytotoxicity. The extracellular LDH in the media is quantified by a series of enzymatic reactions in which LDH catalyzes the conversion of lactate to pyruvate via the reduction of NAD^+ to NADH. Diaphorase uses NADH to reduce a tetrazolium salt to a red formazan product, which can be quantitatively measured at 490 nm. Under defined conditions, such an enzyme reaction to detect extracellular LDH reflects the levels of cytotoxicity.[45] Therefore, the LDH assay is a widely performed cytotoxicity test to evaluate dental materials.[46-48] We also used this assay to investigate the cytotoxicity of provisional resin materials.

Among the five products evaluated, the chemical-activated products (SN, LT, and JE) were more cytotoxic to hDPSCs than were the light-activated (RL) and CAD/CAM-fabricated (VB) products. Chemical-activated PEMA (SN1: ~87%, SN2: ~53%, and SN3: ~52%) and Bis-acryl (LT1: ~78%, LT2: ~65%, and LT3: ~45%) showed higher levels of cytotoxicity with 50% extract concentration than did the chemical-activated PMMA (JE1: ~49%, JE2: ~36%, and JE3: ~2%) when considering cytotoxicity numeric values in the same extract condition ($p<0.05$). Based on the composition of the five tested products, EMA (from SN), Bis-acryl (from LT), MMA (from JE and VB), or UDMA (from RL) is considered a major extractable monomer that induces cytotoxicity in each product. The monomers or eluates released from provisional resin materials were toxic to various types of oral cells, including hDPSCs.[49-51] It was reported that the major substances to induce cytotoxic effects from provisional resin materials consisting of acrylic resin were resin monomers.[50,52] Along with previous cytotoxicity tests, which used

various monomers contained in dental resin materials, EMA and Bis-acryl, which were possibly released from SN and LT, respectively, were more cytotoxic than MMA, which was possibly released from JE.[53,54] This may explain why SN and LT were more cytotoxic to hDPSCs than JE in this study.

Chemical-activated products are older provisional resin materials, which lack biocompatibility and required more than 10 minutes for polymerization.[1,55] Recently, to overcome those characteristics, light-cure resins containing UDMA and CAD/CAM PMMA resins were introduced as provisional resin materials, and they showed enhanced biocompatibility in accordance with this study.[7,56]

Cytokines/chemokines are important mediators of cellular activities and significantly contribute to inflammatory responses.[57] Cytokines such as IL-6 and IL-8 are well known to induce proinflammatory functions.[58,59] Chemokines such as CCL2 and CXCL12 recruit immune reaction-related cells, such as monocytes, memory T cells, or dendritic cells, to the sites of inflammation produced by either pulp tissue injury or infection.[60,61]

In this study, of five types of products, the most cytotoxic provisional resin (SN) at a 50% dilution was chosen to analyze cytokine expression along with the noncytotoxic CAD-CAM acrylic resin block (VB) as a negative control. VB is not thought to increase cytokine/chemokine expression because it is provided in the fully polymerized state from the manufacturer. As an experimental group, SN1, which starts to extract DW from the early set stage, was considered more clinically relevant than SN2 and SN3 in mimicking the impact of provisional resin materials during polymerization. There are concerns about cell death–induced increases in proinflammatory cytokines along with the related mRNA gene expression.[62,63] Therefore, the 12.5% diluted extract from SN (SN1), which first showed less than 30% cell cytotoxicity among serially diluted conditions (50%, 25%, 12.5%, and 6.25%), was selected to analyze cytokine/chemokines to minimize the cell death–induced expression of biomolecules.

The relative cytokine/chemokine expression levels of conditioned media from 12.5% of SN1 and VB eluate-treated hDPSCs were not significantly greater than the control, which suggested that the less cytotoxic concentration (12.5%) of SN1 and VB eluates did not induce a severe inflammatory reaction in hDPSCs. Only MIF and SerpinE1 acted as positive regulators of the inflammatory state, along with the DW-treated negative control. The LPS-treated positive control showed significant expression of the IL-8, CCL2, and CXCL12 proteins.

In summary, within the limitations of this study, extracts from polymerizing (early stage or intermediate stage) provisional resin materials, especially chemical-activated ones (SN, LT, and JE), had more cytotoxicity than the already set counterpart, meaning that exposing a prepared tooth to provisional resin materials in the polymerizing stage could be dangerous to pulp tissue. Otherwise, light-activated ones and CAD/CAM-fabricated resin blocks did not show severe cytotoxicity against hDPSCs (less than 30%) regardless of polymerizing stage, meaning that biocompatibility to pulp tissue is relatively higher in light-activated ones and CAD/CAM-fabricated resin blocks compared to the chemical-activated counterparts. Other adverse effects in terms of inflammation-related cytokine/chemokine expression were not detected under less cytotoxic concentrations of extract in culture media, meaning that a severe inflammatory reaction from hDPSCs is limited after excluding cytotoxicity-induced inflammation. However, dentin-pulp complex characterizations in terms of three-dimensional cellular structure with dentinal tubules, cell diversity in pulp tissue, and three-dimensional extracellular matrix in the dentin-pulp complex were not considered in this study which was a two-dimensional *in vitro* investigation using direct extract treatment. Further investigation using an artificial pulp chamber incubating system with co-culture of various pulp cells or *in vivo* studies is necessary to confirm the above findings.

CONCLUSIONS

It was concluded that extracts obtained from provisional resin materials during polymerization, especially chemical-activated ones (SN, LT, and JE), showed cytotoxic effects on hDPSCs, while they do not induce the expression of proinflammatory cytokines at less cytotoxic concentrations. Therefore, the possible pulp damage from released toxic components should be considered, particularly when chemical-activated provisional resin materials are applied to extensively prepared teeth.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Dankook University Dental Hospital. The approval code for this study is IRB No. H-1407/009/004.

Conflict of Interest

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

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