OPERATIVE DENTISTRY

July/August 2022
Volume 47
Number 4
367-473

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

OPERATIVE DENTISTRY

Volume 47 / Number 4
July/August 2022

www.jopdent.org

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Operative Dentistry (ISSN 0361-7734) is published bimonthly by Operative Dentistry, Indiana University School of Dentistry, Room S411, 1121 West Michigan Street, Indianapolis, IN 46202-5186. Periodicals postage paid at Indianapolis, IN and additional mailing offices. Postmaster: Send address changes to: Operative Dentistry, Indiana University School of Dentistry, Room S411, 1121 West Michigan Street, Indianapolis, IN 46202-5186.

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Clinical Technique/Case Report

A Multidisciplinary Approach to Maxillary Lateral Incisor Agenesis (MLIA): A Case Report

L Lopes-Rocha • D Rocha • T Pinho

Clinical Relevance

To determine the ideal time for insertion of an implant, the state of skeletal growth and emotional state of a young patient must be taken into consideration. However, with the procedure we described, we can achieve a satisfactory conservative solution with a good esthetic outcome and long-term stability.

SUMMARY

Maxillary lateral incisor agenesis (MLIA) is a condition that significantly compromises smile esthetics and is a particular concern in younger patients. The treatment options include orthodontics for space opening with rehabilitation or space closure with canine camouflage. Currently, there is some controversy regarding the most appropriate treatment. In this case report, we propose a multidisciplinary approach through the combination of orthodontic treatment, frenectomy, and a restorative finishing stage with composite resin and dental implants. More specifically, this treatment was planned to orthodontically close the anterior space by opening the premolar area for subsequent placement of implants and enameloplasty with a composite resin.

The replacement of a missing lateral incisor by an implant is a predictable treatment approach, but it might best be deferred until dental maturity and then accurately placed in a well-developed site through a multidisciplinary approach. Precluding the closure of the anterior spaces and the opening of the posterior zone for implant placement, allows for a more stable and appealing esthetic and functional rehabilitation for young patients, in whom esthetic appearance and self-esteem play a primary role.

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http://doi.org/10.2341/21-069-S

INTRODUCTION

The agenesis of one or both maxillary lateral incisors is a frequent clinical condition, affecting approximately 2% of the population.[1,2]Patients with maxillary lateral incisor agenesis (MLIA) are commonly challenged with functional and esthetic problems at a young age, which may affect their confidence and social relationships.[3-5] Restoring an unbalanced dentition is a challenging process, demanding a multidisciplinary approach that should focus on minimally invasive options to satisfy the expected functional and esthetic objectives.[6-9]

Orthodontically, there are two primary treatment options to be considered: 1) space closure with canine camouflage; or 2) space opening with a tooth-supported, resin-bonded fixed dental prosthesis (RBFDP) or dental implant.[10] In cases where the occlusion and anatomy/dimension of the canine in the lateral incisor position are acceptable for camouflage, orthodontic space closure with canine mesialization provides a satisfactory long-term result both functionally and esthetically.[11]A replacement by implant is also a possible solution. The main advantages of this approach are the possibility of obtaining an ideal occlusion, the maintenance of the canine in its natural position, and the clear benefit of avoiding any damage to the adjacent teeth.[12,13] The patient's age is an inexact predictor of dental maturity because young patients develop at different rates.[14] Nonetheless, the placement of implants in the anterior area presents some visible disadvantages, such as bone resorption, infra-occlusion of the implant, gingival retraction, recession of the interdental papillae, gingival changes (including blue staining of the gingiva), and exposure of the abutments.[12,15-18]

In order to avoid such disadvantages, it is possible to open the spaces in posterior sextants, namely in the premolar area. The closure of the anterior space associated with the re-anatomization of the canine into lateral incisor and the first premolar into canine, with the subsequent placement of implants corresponding to a third premolar, is an achievable solution with outcomes that can be as good or superior to those obtained with implants in the anterior sextants.[19]

The objective of this clinical case report is to illustrate the rehabilitation of a patient affected by bilateral MLIA who received an orthodontic treatment comprising anterior space closure and space opening between the premolars. Dental bleaching and rehabilitation of the canines with a direct restorative procedure and dental implants in the premolar area were also performed.

CLINICAL CASE REPORT

A 14-year-old female patient presented with bilateral MLIA associated with bone loss between central incisors and canines. The patient had many concerns about the esthetics of her appearance and was psychologically affected by her smile disharmony (Figure 1). The patient's medical history did not reveal any systemic diseases, and an intraoral examination showed healthy dentition and no symptoms or signs of periodontal disease.

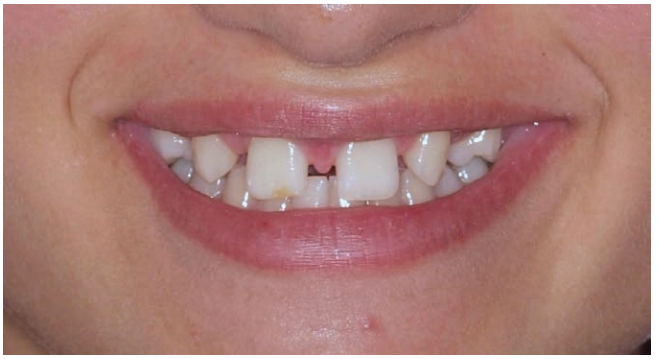

Figure 1. *Smile close-up view before orthodontic treatment.*

In terms of esthetics, the clinical examination of the patient revealed a low smile (considering the patient's young age), competent lips, and a straight profile. Regarding dentition, the examination showed right and left molar Class I and bilateral canine Class II, normal overjet and overbite, first upper premolars in scissor bite relation (Figure 2 A-D); a large maxillary interincisive

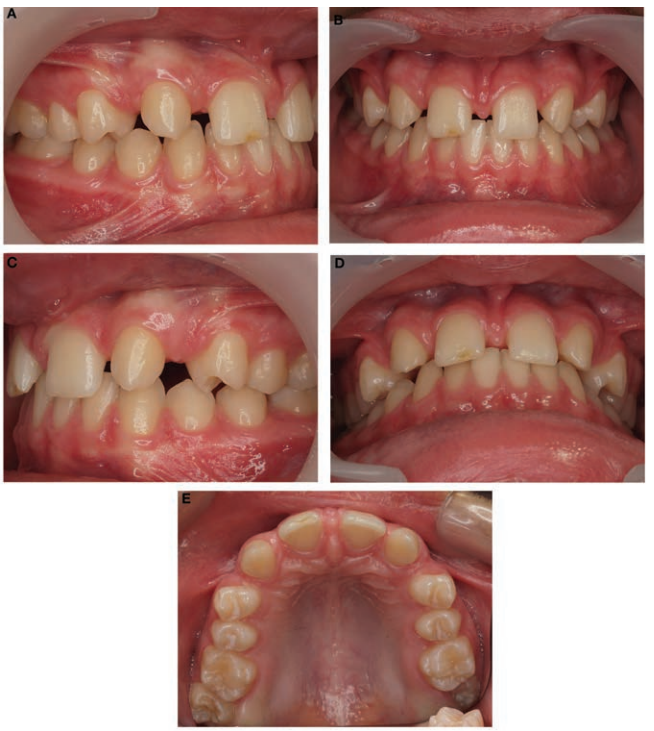

Figure 2. *Preoperative imaging before orthodontic treatment—maxillary lateral incisor agenesis (MLIA).*

true diastema associated with interradicular bone loss and significant atrophy, and a large frenum strongly inserted in the lip and palate (Figure 2E). In addition, the examination revealed a severe hypodivergent biotype, a maxillary dental midline shifted 2 mm to the right in relation to the facial midline, and a slightly negative lower dentomaxillary discrepancy (DDM). The panoramic radiograph showed a congenital absence of maxillary right and left lateral incisors (Figure 3).

First, orthodontic treatment was performed with self-ligated brackets to close the maxillary interincisive diastema and bilaterally close the lateral incisor space with mesialization of the canines. A frenectomy, including the lip and palatal side, was performed (Figure 4). At the end of orthodontic treatment, enameloplasty was performed with dental composite. This treatment allowed for space opening in a more posterior location (between the premolars) for subsequent rehabilitation with implants.

During the orthodontic correction, bite ramps on posterior teeth were necessary for relieving the occlusion, moving the teeth, and correcting dental intercuspation (Figure 5A). Great cooperation was needed from the patient, who was required to use intermaxillary elastics (Figure 5B) throughout the correction. Buttons on lingual surfaces of the first lower premolars with crossed elastics were necessary to correct the scissor bite relation. Coil springs were used between the premolars bilaterally, and they were activated during the correction to enhance mesialization (Figure 5).

During the steel arch wire process (0.19" x 0.25''), some steps were done in three dimensions, with extrusion of the maxillary canines and intrusion of the maxillary first permanent premolars contemplated to improve the gingival architecture and canines' facial-lingual root position (Figure 5B).

The treatment goals of the orthodontic correction were successfully achieved, with anterior space closure and opening of enough space for future placement of implants. At that time, the patient was 17 years old, still too young to place implants between the premolars.

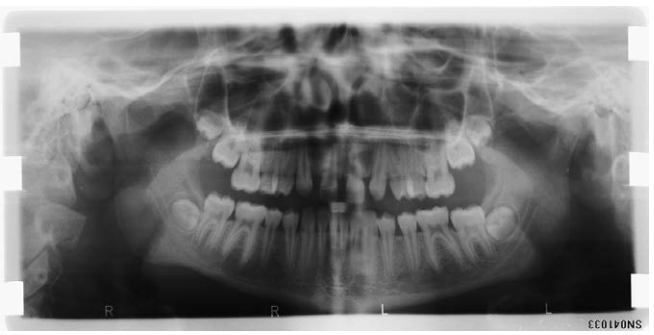

Figure 3. *Initial panoramic radiograph, MLIA.*

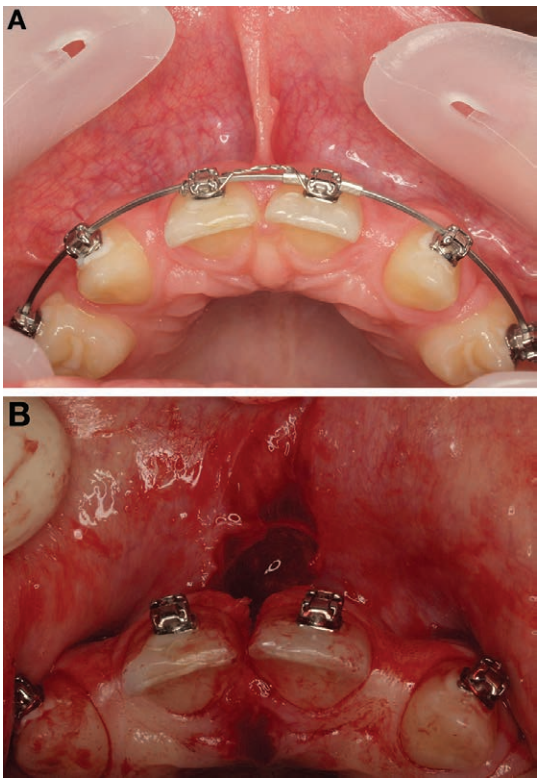

Figure 4. *Preoperative photograph showing high frenal attachment during orthodontic treatment (A) and frenum excised (B).*

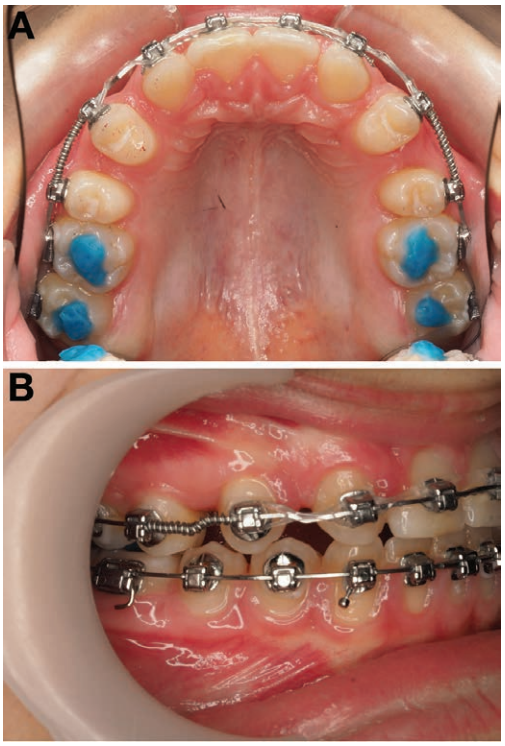

Figure 5. *Orthodontic treatment: (A) Self-ligated brackets with elastic chain and coil spring to close the anterior maxillary spaces; (B) One year later, with wire steps to compensate gingival margins.*

Despite this, the patient was very satisfied with the result and psychologically more confident, as the anterior spaces were closed even though the canines had not yet been remodeled.

To give the patient more occlusal stability and confidence, a removable acrylic maxillary prosthesis with two premolars was provided until the patient had completed skeletal and dental growth and it was possible to place the implants (Figure 6A). In addition, the patient used maxillary and mandibular removable retention, as well as fixed mandibular retention (Figure 6B). At this point, it could be observed that the patient had gingival inflammation (Figure 7), which was controlled mainly through improved oral hygiene habits, an important condition to establish before the dental restorations. However, associated with this inflammation, there was a hypertrophy of the gums that required a gingivectomy prior to the rehabilitation stage.

When the patient was 19 years old, the implants were placed (Figure 8). Considering the clinical observation of the mandibular excursive movements, enameloplasty was attempted on the palatal faces of the mesialized

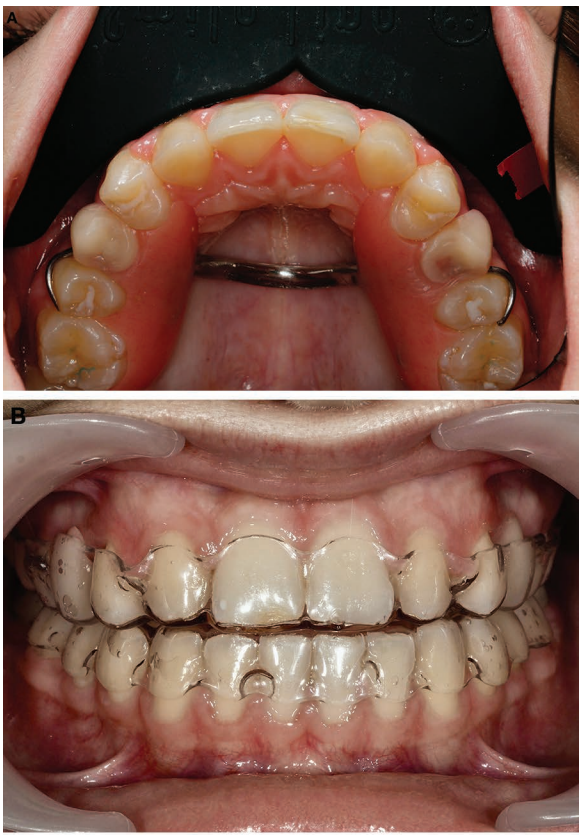

Figure 6. *(A) Placement of removable acrylic maxillary prothesis for esthetic improvement as well as space retention; (B) Maxillary and mandibular removable retention was used to stabilize tooth positions.*

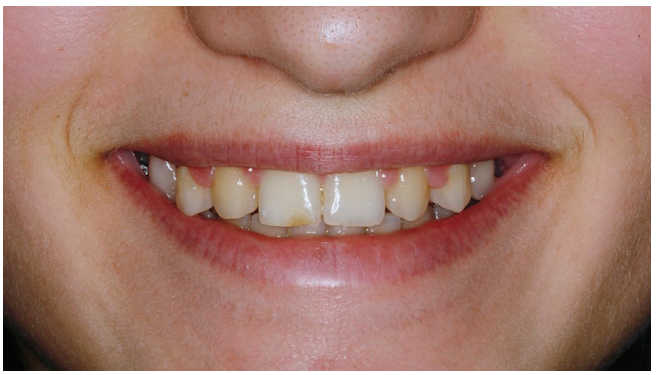

Figure 7. *Smile close-up view after orthodontic treatment with gingival inflammation.*

canines and the palatal cusp of the first premolars. Also, the right central incisor, maxillary canines, and first premolars would be additively remodeled to the shape of lateral incisors and canines, respectively, using direct composite resin. At-home dental bleaching was also included prior to the final restorative phase. This treatment consisted of a two-hour daily regimen of 16% carbamide peroxide (Vivastyle 16%, Ivoclar Vivadent AG, Schaan, Liechtenstein). Four weeks after bleaching, monochromatic restorations were placed on the anterior sextant.

After composite shade selection with the hydrated tooth, rubber dam isolation was used to provide an optimally clean and dry working field. In order to predictably obtain a favorable esthetic outcome with the addition of composite resin, a dimensional guide was fabricated by taking an impression of the palatal surfaces extending over the incisal edges of the maxillary anterior teeth, using an addition silicone putty (Aquasil Soft Putty, Dentsply DeTrey, Konstanz, Germany) from mock-up. The enamel surface was etched with 35% phosphoric acid (Vococid, Voco GmbH, Cuxhaven, Germany) for 30 seconds and rinsed for 20 seconds. Then, a universal adhesive bonding agent was applied (Futurabond U, Voco),

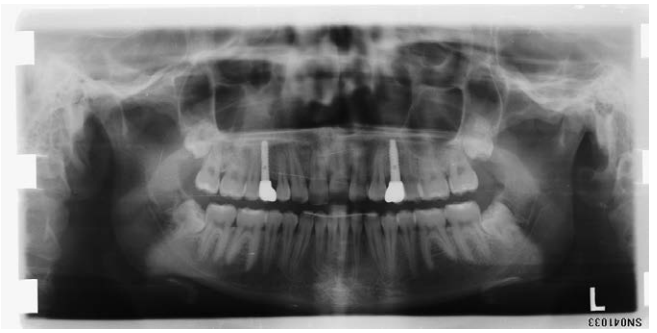

Figure 8. *Radiological investigation after orthodontic treatment and implant placement— panoramic radiograph.*

dried, and polymerized for 20 seconds. The material used for the build-up was a hybrid composite (A1/B1 maxillary central incisors and A2/B2 maxillary canines and first premolars; BRILLIANT EverGlow, Coltène-Whaledent, Altstätten, Switzerland). To prevent the formation of an oxygen-inhibition layer, a glycerin gel was placed on the restoration and polymerized. Any excess material at the margins was removed with a #12 surgical scalpel blade (SwannMorton, Sheffield, England). Finishing and polishing were carried out using fine and extra-fine diamond finishing burs, abrasive disks, and finishing strips (Swiss Flex, Coltene, Cuyahoga Falls, OH/USA). Abrasive silicone points were also utilized (Diamanto, Voco) to obtain better color stability over time and greater wear resistance. Finally, occlusal adjustment was performed.

In the eight-month follow-up, the rehabilitation showed good soft tissue adaptation and excellent esthetic maintenance (Figures 9 and 10).

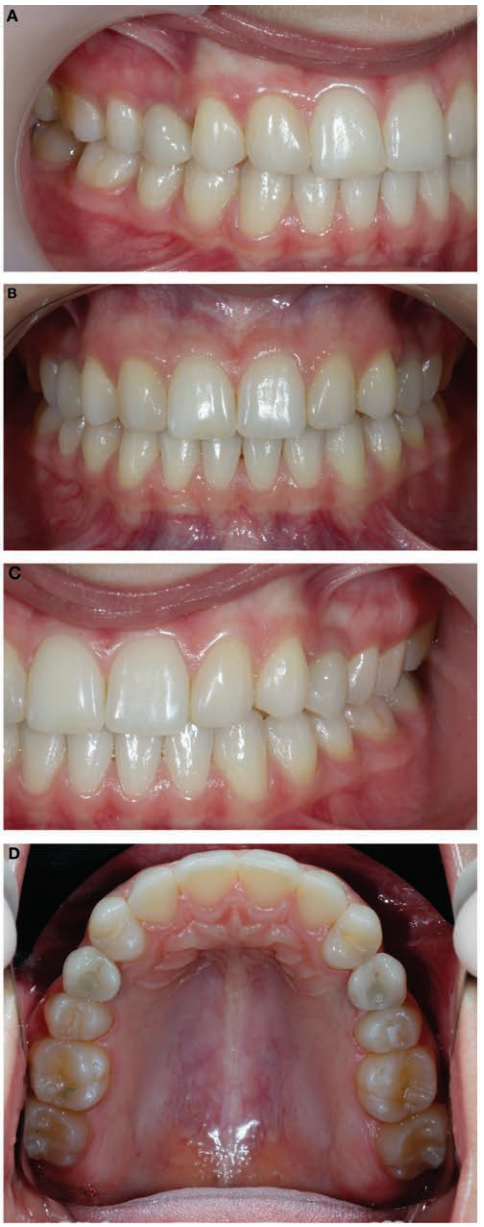

Figure 9. *Intra-oral record after oral rehabilitation with implants and composite buildups (eight- month recall).*

DISCUSSION

The decision to open space in the posterior area was supported by factors such as the patient's young age, the hypodivergent biotype, the presence of interincisor diastemas with bone defects, the anteroinferior crowding, and the Class I molar relationship.

In cases like the present one, in which esthetics was a major concern, it was important to consider not only the position of the teeth but also the gingival architecture. Orthodontic therapy should involve movement of the teeth in three dimensions; this is more specifically the case in the situation of MLIA, where extrusion of the maxillary canine and intrusion of the first permanent premolar will mimic the gingival architecture of a natural smile.[20]In the present case, in spite of the spaces created by the agenesis, the midline diastema was highly associated with a hypertrophic maxillary interincisive frenum.[21] This was surgically removed after the closure of the diastema because it is believed that heavy orthodontic forces deprive the transseptal fibers of sufficient blood supply. In addition, the maintenance of the outcome probably was due to the newly developed tissue, contributing to the good results achieved.[22,23]

Furthermore, orthodontic mesialization allowed bone recovery at the area affected by the agenesis and, with diastema closure, at the interincisive area. This bone recovery minimized the problems associated with subsequent placement of implants at this area, which had had a bone defect. It has been reported that, when compared with natural contralateral teeth, implant-

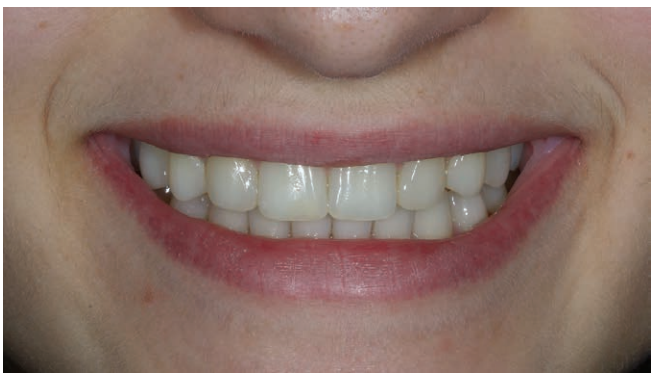

Figure 10. *Smile close-up view after oral rehabilitation with implants and composite buildups (eight- month recall).*

supported crowns replacing upper lateral incisors have shown increased gingival inflammation, increased probing depths, bleeding on probing, and accumulation of plaque.[12,13] Whenever possible and indicated, treatment using anterior space closure is preferable to anterior implants in regard to periodontal health.[12]

The mesial migration of the canine helps to develop the alveolar bone at the place of the congenitally absent lateral incisor, as the bone around the canine will form in the position of the lateral incisor.[24-26] This was taken into consideration during the planning of the orthodontic treatment for this case, and the movements were made in the direction of the bone defects in the interest of bone formation/apposition. Furthermore, it is important that the treatment end during adolescence, to positively impact the individual's self-esteem and social acceptance. The decision to close the anterior spaces was also reinforced by this factor and fortunately did not require a provisional prosthesis in the anterior region before the patient reached the required age to place implants.[27] The authors encourage the use of an maxillary acrylic prosthesis and removable retainers for use at night before placing implants because, as in this case, these measures help to retain tooth position (Figure 6) and provide functional information for the subsequent implant treatment plan.[28,29] Although it is a controversial issue, according to Dietschi and Schatz,[30] implant placement in children younger than 16 to 18 years should be avoided, to prevent infraocclusion due to adjacent alveolar bone growth. Bohner[29] stated that implants must be placed when growth is almost complete. For this reason, the implant surgery on the present case was postponed until the patient reached the age of 19.

In this case, the positioning of the premolars in the place previously occupied by the canines may result in heavy occlusal forces. Occlusion protected by the canine is not possible and this situation can lead to abfraction cervical lesions in the premolars,[12] which must also be re-anatomized for better esthetics and to provide a harmonious smile (Figure 9). However, this substitution is functionally acceptable, giving priority to the occlusion, and consequently distributing the occlusal load between as many posterior teeth as possible.[12] Thus, the opening of the posterior space for implant placement in that region provides an ideal axial load.[19] In this case, the decision to close the space in the anterior area and open the posterior area was taken to eliminate any possibility of bone defect inherent to MLIA and thus eliminate negative esthetic effects in the short and long term.

To optimize smile harmony, before assessing the morphology and proportion, it was important to evaluate the tooth color. Due to their size, canines present a more saturated color when compared with incisors.[31] Hence, following enameloplasty, color correction should be considered to make the teeth brighter, using one of the many available bleaching techniques for vital teeth.[32,33] The restorative procedure was completed four weeks after dental bleaching in order to avoid any possible negative effects of bleaching on bond strength and to allow color stabilization.[34]

The choice of restorative treatment should be based on certain factors that must be well defined, such as preservation of tooth vitality, minimal or no reduction of the dental structure, minimal or no invasion of the gingival area, the esthetic expectations of the patient, cost estimate, and duration of the treatment.[35] In this case, the treatment option chosen was supported by the fact that the canines had a shape and color favorable to space closure, ie, they were small canines with a smaller mesiodistal diameter, and by the fact that a slightly pronounced cusp fits better esthetically and functionally in the position of the lateral incisor. In this way, we were able to solve the problem of the lack of bone in the agenesis area and address the esthetic problem by narrowing spaces in the anterior area during the opening of the space.

The choice of direct restorations with a resin composite over an indirect restoration approach was made mainly due to the fact that the treatment was less expensive and did not involve any injury to the dental tissues.[35-37] Furthermore, the reversible nature of the resin composite technique allows for other treatment approaches in the future. An important benefit of this procedure over others is that the repair may be possible intraorally without the risk of modifying esthetics or mechanical performance.[38] The clinical outcome of anterior resin composite restorations is directly related to the use of a very precise technique, and clinical studies have shown good outcomes without major complications.[36] However, the patient should be mindful that restorations require periodic maintenance because the texture and shade of the material will change over time.[39]

This clinical case demonstrates that a multidisciplinary approach—the combination of initial orthodontic treatment with a restorative finishing stage with composite resin and dental implants, can provide satisfactory esthetic and functional long-term results in a young patient with missing bilateral maxillary lateral incisors.

CONCLUSIONS

In cases of agenesis of the upper lateral incisors, it becomes evident after analyzing the treatment

possibilities that multidisciplinary approaches must be prioritized, linking orthodontics with implantology, prosthodontics, direct restorative dentistry, periodontology, and occlusion. It is important to realize that each patient is unique and needs an appropriate, individualized treatment plan.

Although the usual treatment approach would be the opening of space in the agenesis area, the esthetic limitations in this case resulted in an alternative treatment—the closing of the anterior spaces and opening of the posterior spaces. This solution proved to be viable, showing good results and eliminating any disadvantage of placing implants in the anterior area.

Acknowledgments

This work was supported by IINFACTS—Institute of Research and Advanced Training in Health Sciences and Technologies, in the scope of OrthoAlignPI-4RL-IINFACTS-2019. The rehabilitation with implants was done by José António Fernandes Araújo e Silva.

Conflict of Interest

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

(Accepted 19 June 2021)

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Esthetic Crown Lengthening Surgery – A 30-Year Retrospective and Lessons Learned

JW Robbins

Clinical Relevance

Esthetic crown lengthening surgery is the treatment for altered passive eruption.

SUMMARY

Esthetic crown lengthening (ECL) surgery has been available to our profession for more than 30 years. The objective of this article is to discuss the author's evolution in the techniques associated with ECL surgery. Several technical modifications related to initial incision, ostectomy, and tissue placement will be discussed. In addition, reasons for tissue rebound will be discussed.

INTRODUCTION

Altered passive eruption is a condition that was described by Coslet and others[1] more than 40 years ago. They differentiated these patients into four categories based on the position of the alveolar bone in relation to the cemento-enamel junction (CEJ) and the mucogingival junction. There is much discussion in the literature regarding etiology, diagnosis, and treatment, based on subcategories represented by different names, eg, altered active eruption, delayed passive eruption, and other types and subtypes.[2] However, the clinical treatment of these patients is essentially the same—a full thickness flap with ostectomy. Because the surgeon has no control of the position and thickness of the underlying alveolar bone, a gingivecomy alone is seldom indicated as a treatment for this condition. The one indication for a gingivectomy will be discussed. Therefore, the term altered passive eruption will be used to describe all of the categories of excess gingival coverage of the anatomical crown.

To diagnose a patient with altered passive eruption,[3] two criteria must be met. First, the tooth is short by measurement. The average length of the clinical crown of the normal maxillary central incisor is 10-11 mm. Second, the CEJ cannot be detected in the sulcus with the tip of an explorer. In a patient with a normal attachment apparatus, the CEJ can be detected in the sulcus due to its roughness, compared to the smoothness of the adjacent enamel. However, in the patient with altered passive eruption, the dentist can only feel smooth enamel all the way to the base of the sulcus. The CEJ cannot be felt because it is covered by the attachment apparatus. No anesthesia is required for this exam. The diagnosis can also be made with cone beam computed tomography (CBCT).[4] Allen[5,6] pioneered esthetic crown lengthening (ECL) surgery as the treatment for altered passive eruption.

The author has been performing ECL surgery for 30 years. Over these three decades, my view of this surgical

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http://doi.org/10.2341/21-046-T

procedure has evolved, based primarily on short- and long-term clinical outcomes. It is the purpose of this article to discuss successes, and more importantly, failures, some of which are controllable, and some which are not.

Regardless of the surgical technique, there are three treatment goals. Goal #1 is to thin and move the alveolar bone 2 mm apical to the cementoenamel junction (CEJ) from facial line angle to facial line angle. Some clinicians recommend moving the alveolar bone 3 mm apical to the CEJ to maximize the exposure of the anatomic crown. However, the goal of ECL is to move the bone to the position where nature should have moved it but didn't. In the normal crest attachment, the average distance from CEJ to alveolar crest is 2 mm.[7,8] Additionally, if the alveolar bone is moved 3 mm apical to the CEJ, there is greater likelihood that the patient may have an exposed root surface in the future. The root surfaces should not be scaled after the flap is elevated. Goal #2 is to position the gingival crest 3 mm coronal to the new alveolar crest position. This will result in the CEJ being 1 mm subgingival at the completion of surgery. Goal #3 is to level the tissue at the new position. It has been suggested that a surgical stent is helpful in guiding the ostectomy. This is true with functional crown lengthening surgery. However, in ECL surgery, the postoperative position of the osseous crest is based on the CEJ; therefore, an arbitrarily created surgical stent is not needed.

Some clinicians advocate the use of the laser for a closed ostectomy without flap elevation. McGuire and Scheyer[9] evaluated this technique using an erbium-doped yttrium aluminum garnet (Er:YAG) laser and reported three consistent findings: 1) inadequate ostectomy and thinning of alveolar bone; 2) troughing of alveolar bone; and 3) charring of the root surfaces. For these reasons ECL surgery should be accomplished with an elevated flap.

I performed my first ECL surgical procedure in 1990. Initially, the classic approach of removing a collar of gingival tissue with an internal bevel gingivectomy was used (Figure 1). This initial incision can only be utilized when adequate keratinized tissue will remain after the gingivectomy. A horizontal incision across the midfacial of the papilla was used and the incision was extended to the distal of the second premolars. A full thickness flap was elevated to, or past, the mucogingival junction. Using a high-speed large, round 12-fluted carbide finishing bur, the bone was thinned over the root of each tooth without touching the root with the bur (Figure 2). The bone was also scalloped between the roots to create a normal festooned alveolus. The thinned bone over the root

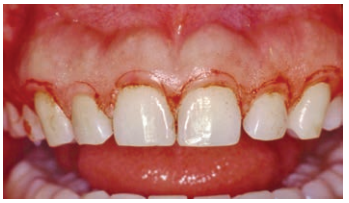


Figure 1. *Internal bevel gingivectomy.*

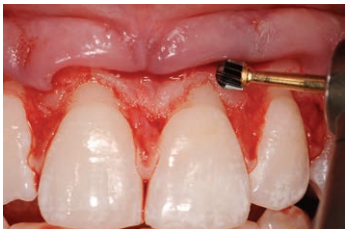


Figure 2. *Thinning and reshaping alveolar bone with large round 12-fluted carbide finishing bur.*

was removed with a 2-mm Wedelstaedt chisel, 2 mm apical to the CEJ from facial line angle to align with the same scallop as the CEJ (Figure 3). This resulted in a small but significant bony ledge where the bone approached the root. Therefore, the final step was to thin the bone to a knife edge with a bullet nosed diamond bur (Figures 4, 5). No attempt was made to create a distal zenith in the alveolar bone. The tissue was then replaced and sutured with simple interrupted sutures using 5/0 chromic gut. The goal of ECL was to uncover most of the anatomic crown, with the CEJ remaining 1 mm subgingival after healing. The sutures were removed two weeks postoperatively.

I experienced two problems with this technique. First, the initial incision was difficult. It was imperative that the gingivectomy be an internal bevel incision so that the marginal tissue would be appropriately thinned. However, it was very difficult to get a uniform thickness of beveled marginal gingiva. In addition, it was essential that the incision over all of the anterior teeth was level and had the same arc. The second problem was that the marginal gingiva commonly healed with a rolled rather than knife edged margin.

For these reasons, I changed to the surgical technique that I have used for most of the last three decades. This technique uses a sulcular incision and apically positioned flap rather than an internal bevel gingivectomy. The initial incision is a sulcular incision (Figure 6) without the removal of a collar of tissue and a thinning incision across the papilla (Figure 7). The same technique for ostectomy is used as before. Finally, the flap is apically positioned so that the gingival crest is 3 mm coronal to the new alveolar crest and sutured in that position with simple interrupted sutures.

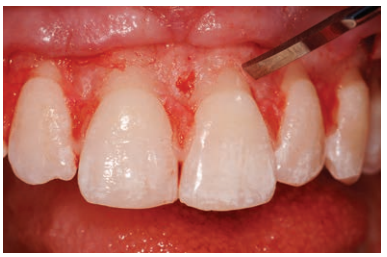


Figure 3. *Removing thinned alveolar bone adjacent to root surface with Wedelstaedt chisel.*

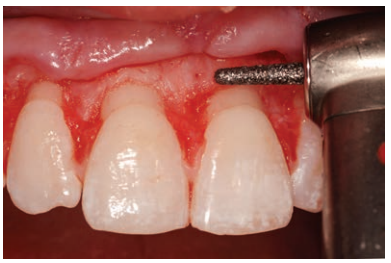


Figure 4. *Thinning of bony ledge created with the chisel, using a bullet nosed diamond bur.*

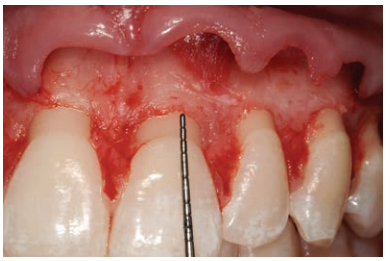


Figure 5. Final ostectomy 2 mm apical to the cemento-enamel junction (CEJ).

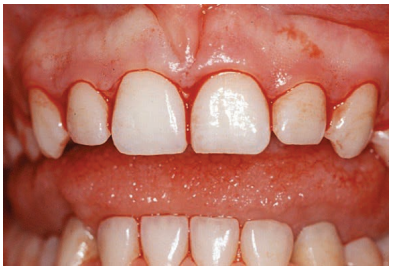


Figure 6. *Sulcular incision.*

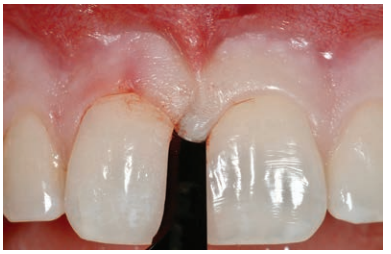


Figure 7. *Thinning incision of the papilla.*

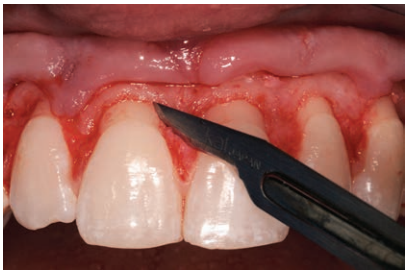


Figure 8. *Reshaping of remaining interproximal papilla to create smooth blend between papilla and flap.*

TECHNIQUE MODIFICATIONS

Over the years, several technique modifications have been implemented based on observation of my surgical outcomes: 1) Stopping the incision at the distal of the second premolars decreases access to the alveolar bone over the second premolar. This commonly results in inadequate ostectomy and a gingival step-down from canine to second premolar, which is unesthetic. Today, the incision is based on the distal extent of the smile, which is usually the mesial of the first molar. The incision should extend one tooth distal to the distal extent of the smile. Therefore, the incision should generally be carried to the mesial of the second molar. 2) The mid-papilla incision commonly left a scar. Currently, the incision is a thinning incision of the papilla with the 15c scalpel blade, essentially parallel to the long axis of the tooth (Figure 7). This results in an almost entire connective tissue bed over the papilla. 3) When the flap is apically positioned, it doesn't blend with the remaining interdental papilla. Therefore, the interdental papilla must be reshaped by narrowing with a 15c scalpel blade to create a perfect blend (Figure 8). 4) When the arc of the ostectomy is parallel to the CEJ, the tissue commonly heals with a gingival contour that is too triangular. In order to prevent this, the ostectomy must be more parallel on the sides and moved slightly more interproximally (Figure 9). This is especially true

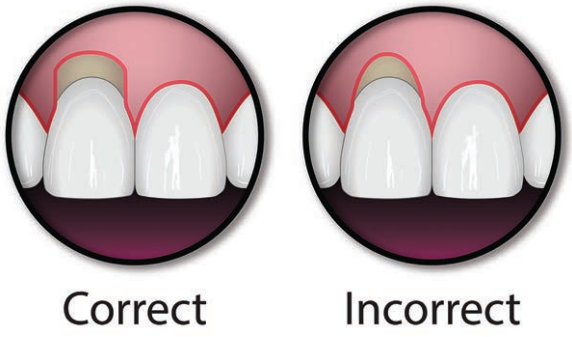


Figure 9. *Correct ostectomy which is more parallel on the sides and moved slightly more interproximally. Incorrect ostectomy which is parallel to the CEJ.*

on the mesial surface of the maxillary lateral incisor, where the ostectomy must be accentuated or the tissue will heal with a very triangular gingival arc (Figures 10, 11). 5) After the ostectomy is complete, the surgeon should stand in front of the patient to evaluate the horizontal symmetry of the newly created alveolar crests, and to ensure the required 2 mm from CEJ to alveolar crest is present. Commonly, this evaluation will reveal the need for additional alveolar recontouring to create the desired symmetry. 6) Chromic gut suture is still an acceptable material; however, 5/0 polyglycolic acid (PGA) suture is easier to use and looks better during the healing phase. The white color of the suture takes the color of blood and is difficult to see at a speaking distance. 7) After completion of the suturing, the surgeon must again stand in front of the patient to ensure that the tissue is level and that there is perfect gingival symmetry. Commonly there is a need for minor gingival recontouring and blending of the papillae and the flap, which is best accomplished with either a laser or electrosurgery. 8) Leaving the sutures for 14 days was found to serve no purpose and slowed healing. Today, the sutures are removed at 5-7 days postoperatively.

POSTOPERATIVE INSTRUCTIONS

The patient is advised not to do anything to activate the upper lip, eg, sucking through a straw, kissing, and/or pulling the lip up to look at the surgical site. For maxillary ECL surgery, the patient is instructed not to brush the entire mouth for four days. On day 5, the patient can start to brush the lower arch, but is instructed not to brush the upper arch for 2 weeks. The patient is given a bottle of antimicrobial rinse and instructed to gently swish and spit twice a day for two weeks.

TISSUE REBOUND

Any surgeon that has done ECL surgery has experienced tissue rebound in a small percentage of patients.[10] There are four reasons for tissue rebound. The first is inadequate ostectomy. This can be either inadequate bone removal apico-coronally and mesio-distally (Figures 10, 11), or inadequate thinning of bone.

The second is bone regrowth over time. The alveolar bone does not regrow coronally; however, in some patients, the thickness of the bone will return, similar to the growth of an exostosis. As the bone becomes thicker, it can cause the gingival crest to migrate coronally. This complication is unpredictable, and there is no way to prevent it.

The third etiology of tissue rebound is orthodontic intrusion of the teeth after the ECL surgery (Figures 12, 13) This is a common occurrence and requires a second-stage surgery at the completion of orthodontics.

The fourth etiology of tissue rebound is a specific type of supracrestal tissue attachment (biologic width). In 1961, Gargiulo and others[7] published their now-classic data on attachment measurements. They reported

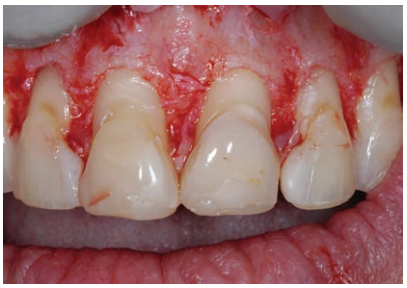


Figure 10. *Inadequate ostectomy on mesial surfaces of both maxillary lateral incisors.*

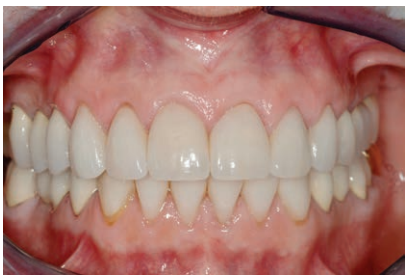


Figure 11. *Clinical healing, demonstrating triangular shaped gingival crests on both maxillary lateral incisors due to inadequate ostectomy on the mesial surfaces.*

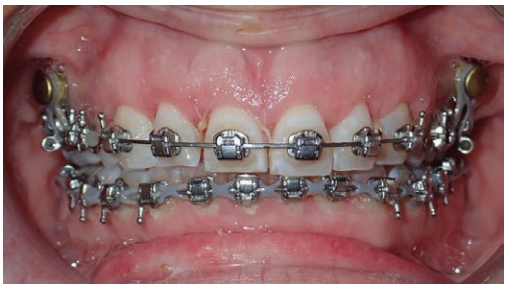


Figure 12. *Esthetic crown lengthening (ECL) surgery, 1 week postoperatively.*

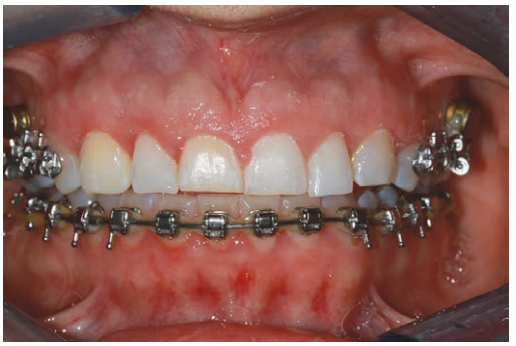


Figure 13. *Tissue rebound after 6 months of orthodontic intrusion.*

the mean measurement of epithelial attachment plus connective tissue attachment was 2.04 mm. In 1977 Ingber and others[11] described "biologic width" and credited Dr D Walter Cohen for coining the term. In 1994, Kois[8] published his classic article on biologic width. He proposed three categories of biologic width based on the total dimension of attachment plus the sulcus depth. In order to operationally define biologic width, Kois suggests that the dentist must determine the total distance from the gingival crest to the alveolar crest. This procedure is termed bone sounding. The patient is anesthetized, and the periodontal probe is placed in the sulcus and pushed through the attachment apparatus until the tip of the probe engages alveolar bone. Based on this measurement from gingival crest to alveolar crest, he proposes three categories of supracrestal tissue attachment—normal crest, high crest, and low crest. By definition, normal crest means that the patient's gingival crest is 3 mm from the alveolar bone midfacial and 3-4.5 mm at the line angles (Figure 14). This occurs approximately 85% of the time. In the high crest patient, the gingival crest is less than 3 mm from the alveolar crest, both mid-facially and at the line angles (Figure 15). This occurs approximately 2% of the time. In the low crest patient, the gingival crest is more than 3 mm from gingival crest to alveolar crest mid-facial and more than 4.5 mm at the line angles (Figure 16). This occurs approximately 13% of the time.

In 2007, the author[12] proposed two different categories of low crest, termed unstable low crest and stable low crest (Figure 17). The unstable low crest patient has a normal attachment apparatus (approximately 2 mm), but a greater than normal sulcus depth. The stable low crest patient has a normal sulcus depth, but a longer than normal attachment apparatus. In order to diagnose a low crest patient as stable or unstable, the dentist must perform gentle sulcus probing in addition to bone sounding. The sulcus probing must be done in a different position than the initial bone sounding.

These two different categories of patients respond very differently to both restorative and surgical procedures. The unstable low crest patient is susceptible to gingival recession, due to the deeper sulcus and unsupported gingival crest. Additionally, this is the only category of biologic width in which a gingivectomy can be successfully used. However, the stable low crest patient is much different, presenting with a normal sulcus depth but having a biologic requirement for a longer attachment apparatus. The stable low crest patient is not susceptible to gingival recession; however, this patient is susceptible to gingival rebound after ECL surgery because the ostectomy is inadequate to provide space for the longer attachment requirement. Therefore, it is necessary for the surgeon to determine the category of biologic width by bone sounding prior to ECL surgery. If the bone sounding reveals that the patient is low crest,

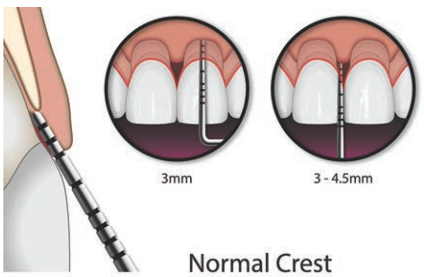


Figure 14. *Normal crest-bone sounding 3 mm from gingival crest to alveolar crest midfacial and 3-4.5 mm from gingival crest to alveolar crest at line angle.*

Figure 15. *High crest—Bone sounding less than 3 mm from gingival crest to alveolar crest midfacial and less than 3 mm from gingival crest to alveolar crest at line angle.*

Figure 16. *Low Crest—Bone sounding greater than 3 mm from gingival crest to alveolar crest midfacial and greater than 4.5 mm from gingival crest to alveolar crest at line angle.*

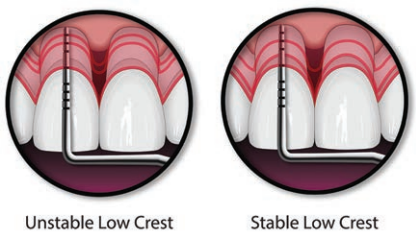


Figure 17. *Unstable low crest. In this illustration, the sulcus depth is 3 mm and the attachment is 2 mm. Stable low crest. In this illustration, the sulcus is 1.5 mm and the attachment is 3.5 mm.*

then the surgeon must determine if the patient is stable or unstable by sulcus probing. If the patient is stable low crest, the surgeon must move the alveolar bone 3 mm apical to the CEJ rather than the traditional 2 mm used in all other biologic width categories (Figure 18). Without the additional ostectomy, there will be gingival rebound at the completion of ECL surgery.

CASE REPORT

A 16-year-old female presented with altered passive eruption (Figures 19, 20), and ECL surgery was performed on the maxillary arch. A sulcular incision was extended from mesial of second molar to mesial of second molar. A full thickness flap was elevated past the mucogingival junction, revealing thick alveolar bone which was too close to the CEJs in many areas (Figure 21). The ostectomy was accomplished, resulting in a 2-mm distance from alveolar crest to CEJ (Figure 22), and the alveolar bone was significantly thinned in posterior quadrants (Figure 23). Closure was accomplished with 5/0 PGA suture (Figure 24). Postoperative healing was uneventful, with a significant improvement in the esthetics of her smile (Figures 25, 26, 27). Seven years after the initial ECL surgery, she returned with a complaint that there had been some tissue rebound in the lateral incisors and the posterior quadrants (Figure 28). A second ECL surgery was accomplished, revealing that the alveolar bone had become significantly thicker adjacent to the lateral incisors and in the posterior quadrants (Figure 29). The alveolar bone was thinned as it had been seven years earlier (Fig 23). Again, the healing was uneventful.

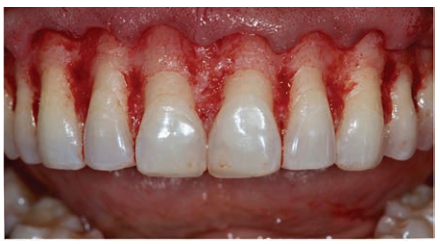
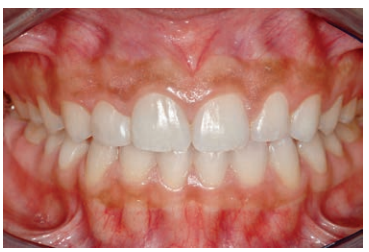


Figure 18. *A low crest stable patient who required 3 mm of ostectomy to create adequate room for their supracrestal tissue attachment requirement.*



Figures 19, 20. *Preoperative photographs.*

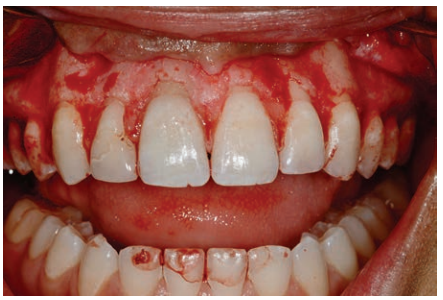
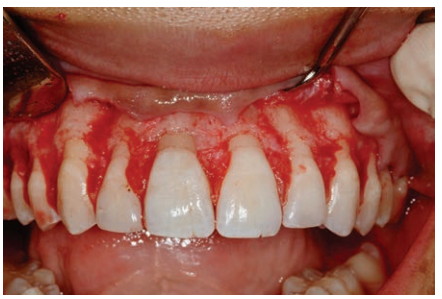
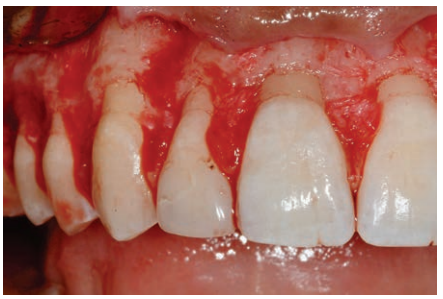


Figure 21. *Pre-ostectomy.*





Figures 22, 23. *Post-ostectomy.*

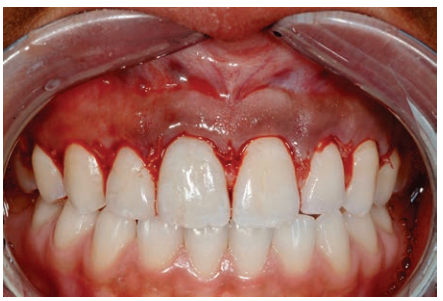
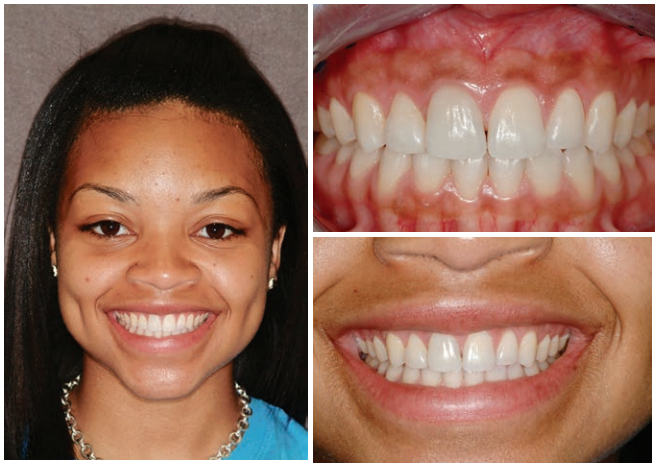


Figure 24. *Postoperative after suturing.*

CONCLUSION

As with all dental procedures, there are commonly several treatment techniques available to the clinician.



Figures 25, 26, 27. *Six weeks postoperatively. Note that the open gingival embrasure between the maxillary central incisors was already present in the surgical photographs.*

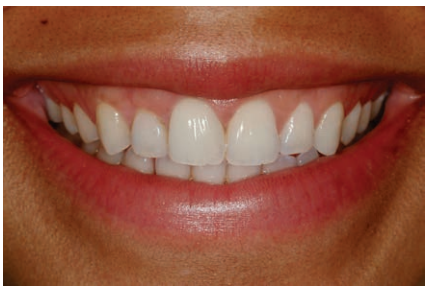


Figure 28. *7-year post-operative photograph demonstrating gingival rebound in maxillary lateral incisors and posterior quadrants.*

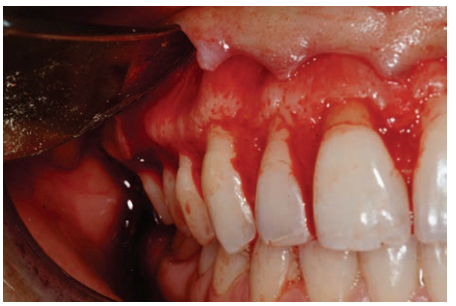


Figure 29. *Second ECL surgery done at 7 years, demonstrating regrowth and thickness of alveolar bone.*

This is certainly true for the treatment of altered passive eruption. It has been the purpose of this article to provide the evolution of my experience with ECL surgery.

Acknowledgments

I would like to acknowledge the three periodontists who have greatly influenced my understanding of periodontal surgery, Dr Alfred Lopez, Dr Eric Rindler, and Dr Pat Allen. I would like to thank Andrew Robbins for the illustrations.

This material was presented by the author at the 2018 annual meeting of the Academy of Operative Dentistry.

Conflict of Interest

The author has no financial interest in any of the companies or products mentioned in this article.

(Accepted 19 June 2021)

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Clinical Research

Twelve-month Clinical Performance Evaluation of a Glass Carbomer Restorative System

ZB Kaynar • N Dönmez

Clinical Relevance

The glass carbomer material claiming to be usable as a permanent restorative material did not exhibit sufficient clinical properties for long-term use as expected when compared with the resin composite material. It is recommended that this material should only be used as a short-term interim restorative material.

SUMMARY

Objective: The aim of this *in vivo* study was to evaluate the clinical one-year follow-up of a silica- and flouroapatite-reinforced glass carbomer filling material as compared to a resin composite restorative material.

Methods and Materials: In this study, a total of 100 restorations were performed. Caries were removed conventionally with diamond burs. Half of the restorations were restored with nanocomposite resin (TEP) (Tokuyama Estelite, Tokuyama Dental, Japan) and the other half were restored with glass carbomer (GC) material (GCP Dental, The Netherlands). Each restorative material was applied according to the manufacturer's instructions. Restorations were evaluated with modified USPHS criteria at the end of the first week, 6 months, and 12 months. Data were analyzed using Fisher's Exact Chi-Square test, Fisher Freeman Halton Test, and Continuity (Yates) Correction. The Wilcoxon sign test was used for intra-group comparisons of the parameters.

Results: When the filling materials were compared with one another, a statistically significant difference was observed in the 12th month on the marginal discoloration. A statistically significant difference was observed between the two materials in the 6th month on the marginal adaptation (p<0.05).

Conclusions: In view of these results, there is a need to improve the physical properties of the GC filling material in further *in vivo* studies.

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http://doi.org/10.2341/21-016-C

INTRODUCTION

Glass ionomer cements (GIC) have become one of the most widely used materials in dentistry since the

1970s.[1] Properties of being able to chemically bond to dental hard tissues, showing anticariogenic properties, releasing fluoride, and having an expansion coefficient close to dentin have made the use of GICs widespread. Despite all these advantages, their disadvantages—such as poor compressive, tensile strengths, and aesthetic properties; low fracture and wear resistance; the inability to eliminate microleakage; short working time; and a long-lasting hardening process—have led to studies to improve the material.[2] Glass carbomer cement (GC) (GCP Dental, Netherlands) is one of the materials that developed as a result of studies on the improvement of GICs.

Although GC is considered a glass ionomer-based material, the presence of nano-sized powder particles and fluorapatite distinguish GCs from GICs. In the development of this material, the aim is to create an enamel-like structure using nanoparticle technology.[3,4] There are nano-sized fluorapatite/hydroxyapatite particles in the content of GCs. The addition of nano-hydroxyapatite and nano-fluorapatite is known to increase the mechanical properties of glass ionomers and their bond strength to dentin.[5] The reactive glasses inside them are modified with dialkyl siloxanes and the liquid of GC consists of weak polyacrylic acid and does not contain resin, solvent, and monomer.[6,7] With the addition of fluorapatite, the GIC is converted to a material similar to fluorapatite.[8] Furthermore, thanks to the fine structure of cement, a smooth and polished surface similar to resin composites has been obtained.

GC is used together with an organic, biocompatible surface coating gloss (GCP Gloss, GCP Dental) that is carbon-silicone based. The gloss aids in producing an excellent restorative material by improving the transparency which is necessary for optimum heat-based setting. It also maintains the restorative material from moisture and saliva contamination during the initial setting phase, and from dehydration later on.[8] The monomer-free condition and the addition of nano-sized hydroxyapatite and fluorapatite particles in GC ensures it as a more biocompatible option than RMGIC.[9]

Similar to GICs, GCs are also chemically hardened. Manufacturers have recommended that the wear resistance and compressive strength of the material is increased through the use of a light device with a high light power during the hardening process of GC.

Although the mechanical and physical properties of the GC restorative system have been studied by laboratory studies in the literature, there is a limited number of studies investigating the clinical performance of this material. Therefore, the aim of this randomized controlled clinical trial was to compare the clinical performance of GC with a nanohybrid posterior resin composite (TEP) (Tokuyama Estelite Posterior, Tokyo, Japan) in the restoration of Class II and Class I cavities and to evaluate the clinical performance for 12 months. The hypothesis of the study was that both restorative materials would have similar clinical performance.

METHODS AND MATERIALS

Study Design

This study was a randomized controlled clinical trial where teeth were randomly assigned to one of the two restorative material groups with an allocation ratio 1:1.

This clinical study was approved by the Clinical Research Ethics Committee. Patients were informed about the purpose of the study, treatment strategies, dental materials to be used, risks of treatment, and written consents were taken before beginning the study. The study was registered at Clinical Trials.gov Protocol Registration and Results System with the ID: NCT04127929 (16.10.2019). PASS Sample Size Software (NCSS, LLC, Kaysville, Utah) was used to calculate the sample size. In order to get the f = 0.25 effect difference between the groups with 80% power and an alpha error of 5%, at least 50 restorations per group were needed.

The samples in this study were allocated similar to the clinical study by Baba MG and others.[10] The study sample consisted of 100 premolar/molar teeth in healthy, cooperative patients with the following eligibility criteria: patient (26 female, 10 male) between the ages of 20 and 25 years (mean age: 23 years) with a proximal and occlusal lesion on at least one premolar or molar who were available for follow-up after 1 week, 6 months, and 12 months of restoration placement. All patients were recruited from the Restorative Dentistry Clinic in the Faculty of Dentistry, from October 2017 until April 2019.

Inclusion and Exclusion Criteria

The inclusion criteria were: (a) no systemic disorders; (b) older than 18 years of age (20-25 years of age); (c) presence of vital molar/premolar teeth with occlusal or proximal caries; (d) no parafunctional habits such as grinding or clenching of the teeth; (e) no sensitivity to percussion; (f) no spontaneous pain; (g) no luxation; (h) having good cooperation; and (i) having agreed to attend regular follow-up evaluations. The exclusion criteria were: (a) presence of any indication for endodontic treatment or extraction (abscess, swelling, fistula, pain on palpation or percussion, spontaneous pain or night pain); (b) teeth with a congenital developmental defect; (c) teeth with pathological mobility; (d) patients under the age of 18; (e) teeth which do not have normal

Table 1: *Description of Experimental Materials*

Restorative Material	Type	Manufacturer	Composition	Lot No	Used Color
Estelite posterior composite resin	Nano-hybrid composite	Tokuyama Dental, Tokyo, Japan	Organic matrix; Bis-GMA TEGDMA Bis-MPEPP Inorganic; Silica-zirconia Particle size:2 um Particle size/ratio: 0.1-10 um Weight;%84 filler Volume;%70 filler	243E67	A 2
Glass carbomer	Glass-ionomer based	GCP Dental, Netherlands	Nano-fluoroapatite Nano-hydroxyapatite Polyacids Fluoroaluminosilicate glass	7609020	A 2
Glass carbomer bloss	Silicon based	GCP Dental, Netherlands	Modified polysiloxanes	1607101	

Abbreviations: Bis-GMA, bisphenol A-glycidyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; Bis-MPEPP: 2,2-bis[(4-methacryloxy polyethoxy)phenyl]propane.

occlusion due to skeletal or pathological reasons; and (f) loss of contact or opposing tooth.

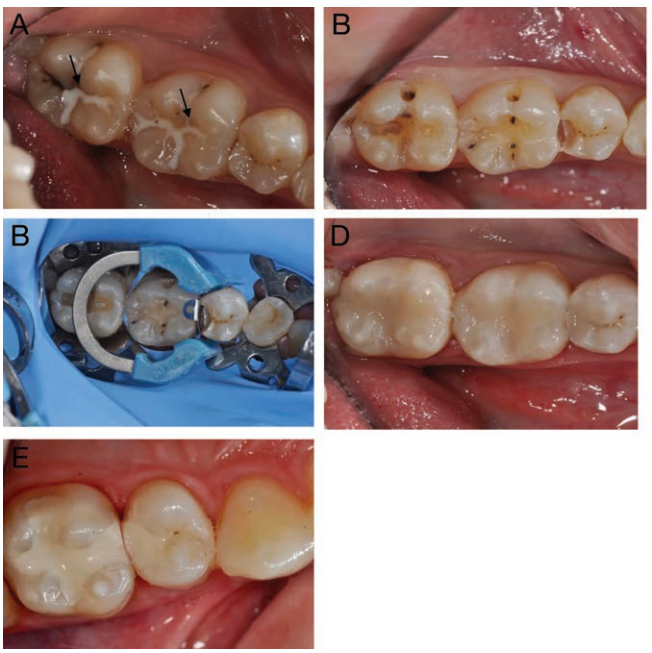

Figure 1. *Each stage of restoration procedures. (A): The view of carious lesions. (B): The view of teeth after removal of caries. (C): The cavity isolation with rubber-dam. (D): The finishing of composite restorations and control with articulation paper. (E): The finishing of GC restorations. Abbreviations: GC, glass carbomer.*

Lesion Selection

A total of 100 Class I (54) and Class II (46) (MO or OD) carious lesions at levels of D1 or D2 (according to clinical and radiographic evaluations) with a minimum of two and a maximum of four permanent premolars or molars according to International Caries Detection and Assessment System (ICDAS)[11] were included in the study.

Randomization and Allocation

The included teeth were assigned randomly by the second author blindly, using the "bowl technique," to one of the two restorative material groups.

According to type of restorative material to be applied, patients included in the study were randomly divided into two groups. Group 1: Nano-hybrid composite restoration group (Class I: 28; Class II: 22); Group 2: Glass carbomer restoration group (Class I: 26; Class II: 24). Two different restorative materials were used in this study (Table 1).

Restorative Procedure

The same experienced dentist performed all restorative procedures. Routine professional oral care, including dental surface cleaning and oral hygiene motivation, were performed. The initial photos of the teeth were taken using a digital camera (Nikon D7200, Tokyo, Japan) with the help of an intraoral photo mirror.

Table 2: Modified USPHS Criteria

Criterias	Scores	Explanations
Retention	Alfa	No loss of restorative material
	Charlie	Any loss of restorative material
Color match	Alfa	Matches tooth
	Bravo	Acceptable mismatch
	Charlie	Unacceptable mismatch
Marginal discoloration	Alfa	No discoloration
	Bravo	Discoloration without axial penetration
	Charlie	Discoloration with penetration indirection pulpal
Anatomic form	Alfa	Continuous
	Bravo	Slight discontinuity, clinically acceptable
	Charlie	Discontinuous, failure
Marginal adaptation	Alfa	Closely adapted, no crevice is visible
	Bravo	Crevice is visible, explorer will penetrate
	Charlie	Crevice in which dentin is exposed
Secondary caries	Alfa	No caries present
	Charlie	Caries present
Postoperative sensitivity	Alfa	Not present
	Bravo	Sensitivity with diminishing intensity
	Charlie	Constant sensitivity without diminishing intensity
Surface texture	Alfa	Enamel-like surface
	Bravo	Surface rougher than enamel, clinically acceptable
	Charlie	Surface unacceptably rough

Abbreviations: USPHS, United States Public Health Service.

Local anesthesia (Ultracain DS Fort, Sanofi Health Products, Istanbul, Turkey) was performed depending on the patient's needs. The removal of caries on the occlusal and proximal surfaces of the teeth was started using rotary instruments (W&H, Austria) and diamond burs (G&Z Instruments, Austria). A steel bur was used to remove caries in dentin tissue. Cavities were prepared in accordance with the minimally invasive approach.

Before restoration of the teeth, cavity isolation was provided with rubber-dam, cotton rolls, and saliva suction for both materials. The sectional matrix system (Palodent V3, Dentsply, USA) was used to create a contact in Class II cavities. For the resin composite restoration group, in both cavity types (Class I and Class II), enamel edges were roughened by 35% orthophosphoric acid for 30 seconds using a selective etch method. After rinsing and drying procedures, two-step self-etch adhesive system (Clearfil SE Bond, Kuraray, Japan) was applied in both cavity types (Class I and Class II) where composite material would be applied. The cavities were restored using both restorative materials according to the manufacturer's instructions. In Group 1, composite material was incrementally applied in the cavities and light cured with a Light Emitting Diode light curing unit (VALO Cordless, Ultradent, USA) set at a standard power of 1000 mW/cm^2. For Group 2, etching and bonding procedures were not applied and the glass carbomer material was placed in the cavity in a single stage. After the cavity was completely filled, the surface cover with silicone content was applied to the restoration and condensed with finger pressure. After that, the restoration was cured for 60 seconds using the GCP CarboLED (GCP Dental), which is a thermo-cure, high-energy lamp that operates on wavelengths higher than those produced by regular light-cure devices (1400 mW/cm^2).

After removing the rubber dam, occlusion control, finishing, and polishing were done with fine grain, yellow band, end flame-shaped diamond burs (G&Z Instruments, Austria). The restorations were polished under water cooling using polishing pastes containing diamond particles (Kuraray Twist Dia, Japan). Surface cover was applied again, following the finishing and

polishing procedures. All restorative procedure steps are shown in Figure 1.

Clinical Evaluation

The restorations were evaluated clinically with 1 week, and subsequently at 6-month and 12-month follow-ups. The clinical evaluation was performed by two calibrated observers other than the clinician who placed the restorations using modified United States Public Health Service (USPHS) criteria (Table 2).[12] Restorations were scored using the terms Alpha, Bravo, and Charlie. Alpha was used for restorations that were considered clinically successful; Bravo was used for the restorations with several deficiencies but requiring no replacement; and Charlie was used for the clinically unacceptable restorations where the restoration had to be replaced.[13] In case of a disagreement, a consensus between examiners was achieved after discussion. Prior to the study, calibration was performed on e-calib between the two observers.

Statistical Analysis

Statistical analysis was performed with SPSS Statistics software, Version 22. Fisher's Exact Chi-Square test, Fisher Freeman Halton Test, and Continuity (Yates) Correction were used to compare qualitative data. The Wilcoxon sign test was used for intragroup comparisons of parameters. The level of significance was set at $\alpha = 0.05$.

RESULTS

A flow diagram is presented in Figure 2. After 12 months, 100 restorations in 36 patients were evaluated and scored according to the USPHS criteria. The overall clinical recall rate of restorations at the 12-month recall was 100%. The clinical properties of the restorations were evaluated according to the CONSORT flow diagram. The modified USHPS scores of the restorations are given in Table 3.

When the Charlie score was observed for a clinical evaluation criteria, the restorations were replaced. In terms of anatomical form, marginal adaptation, and retention, Charlie was scored in some restorations of the GC group. Restorations were replaced with the GC.

Retention

No statistically significant difference was found between the 1-week, 6-month, and 12-month performance results for either restorative material group in terms of retention. When materials were compared with different periods in terms of retention, there was no statistically significant change in terms of the Alpha score after 6 and 12 months in the TEP group compared to the 1

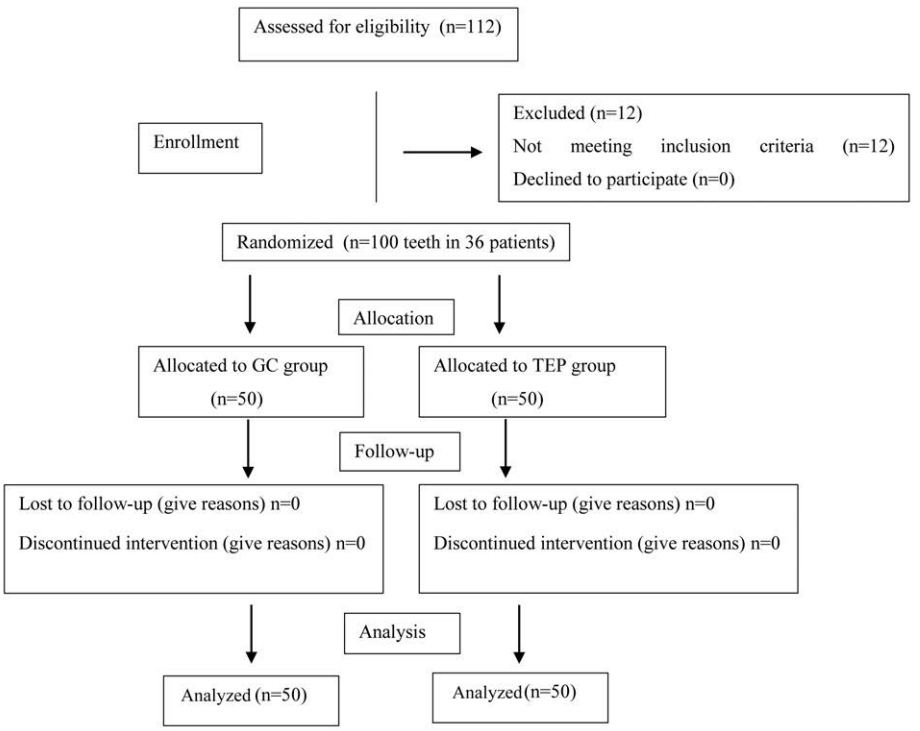

Figure 2. *Flow-chart of 12-month follow-up with GC and TEP restorations. Adapted from CONSORT flow diagram. Abbreviations: GC, glass carbomer; TEP, Tokuyama Estelite Posterior restorations.*

Table 3: *Baseline, 1-week, 6-month, and 12-month Clinical Evaluation of Restorations According to USPHS Criteria*

		GC *n* (%)	TEP *n* (%)	*p*	GC *n* (%)	TEP *n* (%)	*p*	GC *n* (%)	TEP *n* (%)	*p*
Retention	Alfa	50 (100)	50 (100)		48 (96)	50 (100)	0.45	46 (92)	50 (100)	0.12
	Charlie				2 (4)	0 (0)		4 (8)	0 (0)	
Color match	Alfa	50 (100)	50 (100)		45 (90)	50 (100)	0.06[a]	43 (86)	48 (96)	0.19[b]
	Bravo				5 (10)	0 (0)		5 (10)	2 (4)	
	Charlie							2 (4)	0 (0)	
Marginal	Alfa	45 (90)	50 (100)	0.06[a]	42 (84)	50 (100)	0.01*	35 (70)	45 (90)	0.014[b,*]
Adaptation	Bravo	5 (0)	0 (0)		8 (16)	0 (0)		10 (20)	5 (10)	
	Charlie							5 (10)	0 (0)	
Anatomic form	Alfa	50 (100)	50 (100)		40 (80)	45 (90)		37 (74)	43 (86)	
	Bravo				8 (16)	5 (10)		9 (74)	4 (8)	
	Charlie				2 (4)	0 (0)		4 (8)	3 (6)	
Marginal	Alfa	50 (100)	50 (100)		48 (96)	48 (96)	1.000[a]	37 (74)	50 (100)	0.000[b,*]
Discoloration	Bravo				2 (4)	2 (4)		6 (12)	0 (0)	
	Charlie							7 (14)	0 (0)	
Secondary	Alfa	50 (100)	50 (100)		50 (100)	50 (100)		50 (100)	50 (100)	
Caries										
Post-operative	Alfa	46 (92)	48 (96)		48 (96)	50 (0)		48 (96)	50 (0)	
Sensitivity	Bravo	4 (8)	2 (4)		2 (4)	0 (0)		2 (4)	0 (0)	
Surface	Alfa	50 (100)	50 (100)		43 (86)	45 (90)	0.76[a]	40 (80)	42 (84)	0.311[b]
Texture	Bravo				7 (14)	5 (10)		7 (14)	8 (16)	
	Charlie							3 (6)		

Abbreviations: USPHS, United States Public Health Service.
[a]*Fisher exact test.*
[b]*Wilcoxon sign test.*
*p<*0.05.*

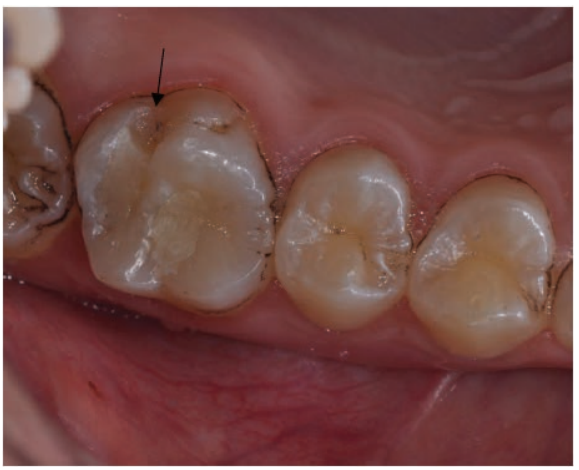

Figure 3. *Restoration scored as Charlie because of loss of retention on palatal surface of GC restoration. Abbreviations: GC, glass carbomer.*

week score (p>0.05). In GC, there was a statistically significant increase in the Charlie score at 12-months (8%) compared to 1 week (0%) (Figure 3).

Surface Texture Change

There was no statistically significant difference between the two restorative materials in terms of surface texture changes after 1 week, 6 months, and 12 months. When materials were compared with different periods in terms of surface texture change, the Bravo scores for surface texture change at 6 months (10%) and 12 months (16%) in the TEP group were found to be statistically significantly higher compared to after 1 week (0%) (p=0.05). In GC, Bravo scores for surface texture change at 6 months (14%) and 12 months (14%) were found to be statistically significantly higher compared to 1 week (0%) (p<0.05).

Color Match

There was no statistically significant difference between the two restorative materials in terms of color match at one-week, six-months and 12-months.

When materials were compared with different periods in terms of color match in the TEP group, there was no statistically significant change in terms of color match at 6 months and 12 months compared to 1 week. Also in the TEP group, there was no statistically significant change in terms of color match after 12 months compared to 6 months ($p>0.05$). In GC, Bravo scores for color match at 6 months (10%) and 12 months (10%) were found to be statistically significantly higher compared to the 1 week (0%) ($p<0.05$). There was no statistically significant change in terms of color match results at 12 months compared to 6 months ($p>0.05$).

Marginal Discoloration

There was no statistically significant difference between the two restorative materials in terms of marginal discoloration at 1 week and 12 months. A statistically significant difference was observed between the two restorative material groups in terms of discoloration at 12 months ($p<0.05$). In the GC group, Bravo and Charlie scores for marginal discoloration at 12 months (12% and 14%, respectively) were found to be statistically significantly higher compared to the 1 week (0% and 0%, respectively) ($p<0.05$). Bravo and Charlie scores for marginal discoloration at 12 months (12% and 14%, respectively) were found to be statistically significantly higher compared to 6 months (4% and 0%, respectively) ($p<0.05$) (Figure 4).

Anatomic Form

There was no statistically significant difference between the two restorative materials in terms of anatomic form at 1 week, 6 months, and 12 months. In the TEP group, Bravo scores for anatomic form at 6 months (10%) and 12 months (8%) were found to be statistically significantly higher compared to 1 week (0%) ($p<0.05$).

In the GC group, Bravo and Charlie scores for anatomic form at 6 months (16% and 4%, respectively) and 12 months (18% and 8%, respectively) were found to be statistically significantly higher compared to 1 week (0% and 0%, respectively) ($p<0.05$).

Marginal Adaptation

When both restorative material groups were evaluated in terms of marginal adaptation, the percentages of Alpha scores for marginal adaptation were 100% in the TEP group and 90% in the glass carbomer group at 1 week. Although the difference between them was close to the significance level, no statistically significant difference was observed between them ($p>0.05$). Percentages of Bravo scores for marginal adaptation was 16% and 0% in the GC and TEP groups at 6 months, respectively. There was a statistically significant difference between the groups in terms of Bravo scores for marginal adaptation ($p=0.006$; $p<0.05$). In the TEP group, the Bravo scores at 12 months (10%) were found to be statistically significantly higher compared to 1 week (0%) ($p=0.025$; $p<0.05$). A statistically significant increase was observed between the 6-month and 12-month Bravo scores (0% and 10%, respectively) ($p<0.05$). In the GC group, there was a statistically significant increase in the Bravo score at the 12-months (16%) compared to the first week (10%) ($p<0.05$). Bravo and Charlie scores

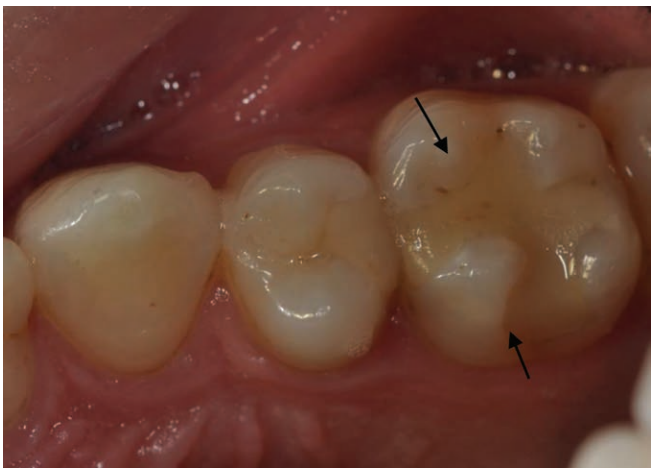

Figure 4. *Restoration scored as Charlie because of marginal discoloration of GC restorations. Abbreviations: GC, glass carbomer.*

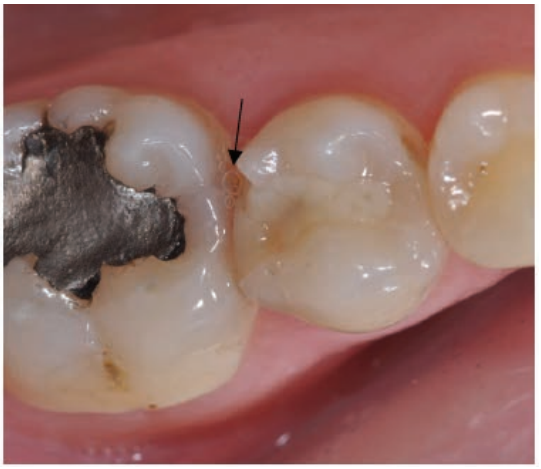

Figure 5. *Restoration scored as Charlie because of marginal fracture in the GC group at the end of 12 months. Abbreviations: GC, glass carbomer.*

at 12 months (20% and 10%, respectively) were found to be statistically significantly higher compared to six months (16% and 0%, respectively) ($p<0.05$) (Figure 5).

Secondary Caries

No secondary caries were observed in either group at 1 week, 6 months, and 12 months.

Postoperative Sensitivity

No statistically significant difference was observed between the materials in terms of postoperative sensitivity at 1 week, 6 months, and 12 months.

DISCUSSION

Although a large number of laboratory studies have been conducted on this new GC material in recent years, the results of studies evaluating the clinical success of this material have not been clear when used as a permanent restorative material in adult individuals. The present clinical study evaluated the clinical performance of the GC filling material used in adults as a permanent restorative material. At the end of the study, statistically significant differences were observed between the materials in terms of marginal discoloration, marginal adaptation, anatomic form, and retention. Therefore, our hypothesis was partially rejected.

Today, resin composite materials are the most preferred restorative materials in the restoration of the posterior and anterior teeth. Resin composites show shrinkage during polymerization, leading to several disadvantages including microleakage, deterioration of marginal adaptation, marginal fractures, postoperative sensitivity, and development of secondary caries. Glass carbomer filling material, one of the glass ionomer-based restorative materials developed in recent years, has been introduced as an alternative restorative material to resin composites.

The literature review showed that there were no clinical studies in which GC was used as a restorative material in adults, however, there were studies where it was used as a fissure sealant. In a study by Gorseta and others,[9] glass carbomer and resin-based fissure sealant material were used as fissure sealants and 100% clinical success was achieved in both materials in terms of retention at 6 months, however, this rate decreased to 75% at 12 months, but it was not statistically significant. In a four-year clinical follow-up study by Zhang and others where high viscosity GIC, GC, and resin-based fissure sealant were used as fissure sealants, the GC group was found to be less successful in clinical practice compared to other materials.[14]

El-Housseiny and others[15] concluded in their study that glass carbomer restorations showed significantly worse clinical performance than resin-modified glass ionomer and composite restoration in first primary molars in terms of anatomical form and marginal adaptation. These results are similar to our study.

In a three-year clinical follow-up study by Hu and others when glass carbomer fissure sealant, resin-based fissure sealant, and glass ionomer fissure sealant were used, no significant differences were observed between the materials in terms of pit and fissure retention rate.[16]

Chen and others conducted a study in which they followed the anti-caries effects of glass ionomer, GC and resin-based fissure sealants for six months, one year and two years and found that the lowest retention rate was in the GC group at the end of two years.[17]

In a study by Olegario and others, GC, high viscosity glass ionomer, and compomer material were clinically monitored for three years using an atraumatic restorative technique and the clinical success of the GC material was found to be significantly lower than that of compomer and high viscosity GIC material.[18]

In the present study, there was no statistical difference between 1 week and 6 months in the glass carbomer group, however, there was a statistically significant increase in the 12 month Charlie score compared to the 1 week score ($p=0.046$; $p<0.05$). This finding was similar to the findings reported by Olegario and others.[18]

As with GIC materials, it is recommended to use a silicone-based sealant to protect the surface from moisture and saliva for GC restorations.[8] In a laboratory study by Zoergiebl and others,[19] sealant application was reported to have no effect on the mechanical properties of GCs. On the other hand, Menne-Happ and others[8] reported in their laboratory study that the sealant applied to the glass carbomer protected the surface of the material from dehydration and made finishing and polishing processes easier. Menne-Happ and others[8] compared the groups that applied sealant to those that did not and reported that surface cracks were formed in the group in which no sealant was used when glass carbomer samples were examined visually. This was attributed to the dehydration due to not using any sealant. In the present clinical study, a silicone-based sealant was applied both to facilitate condensation of the material and to protect it from dehydration. Following the sealant application, light was applied for 60 seconds. There was no statistically significant difference between the materials at 1 week, 6 months, and 12 months when the cavities were restored with both materials in terms of surface texture. This may be due to the use of the silicone-based sealant on the surface of the glass carbomer material.

No statistically significant difference was found between the TEP and GC groups in terms of marginal adaptation at 1 week. However, there was a statistically significant difference between TEP and GC groups only in terms of 6 month Bravo scores (p=0.006; p<0.05). However, percentages of Alpha, Bravo, and Charlie scores of the GC group were 70%, 20%, and 10%, respectively, after 12 months. Therefore, a statistically significant difference was observed between the two groups in this regard (p=0.014; p<0.05). Although there was no polymerization shrinkage in the glass carbomer material unlike the resin composite, significantly lower values were obtained in terms of marginal adaptation. This may be due to the fact that GC was less resistant to occlusal forces than the resin composite (84% filler by weight and 70% filler by volume). This result was compatible with the findings reported in the 6 month clinical follow-up study by Glavino and others[20] who used GC as a fissure sealant.

Secondary caries formation, incidence of which is directly proportional to follow-up period, is one of the criteria to consider for evaluating the clinical success of restorations.[21] Some clinicians suggest that a 4-6 year follow-up is needed to determine the clinical success of any restoration.[22] In this study, no statistically significant difference was found between the restorative materials in terms of secondary caries formation. This may be due to the fact that the clinical follow-up period was limited to 12 months. Also, the presence of silicate and fluoride in the content of GC may be one of the factors preventing secondary caries formation.

In clinical practice, nanohybrid composites are preferred because these materials have strong mechanical properties, similar to hybrid composites, and also have good polishing properties similar to microfill composites.[23] When the teeth restored with TEP were evaluated for color matching, a Bravo score was obtained in only two restorations at the end of the 12 months. The high success rate (96% Alpha, 4% Bravo) may be due to the high polishing feature of nanohybrid composites.

Considering the marginal discoloration results in the GC group at the end of 12 months, the TEP group was observed to have 100% Alpha scores whereas the GC group had 14% Charlie scores where restorations were required to be replaced. This may be attributed to the fact that GC materials are less resistant to masticatory forces than TEP. Although these results were obtained after a 1-year clinical follow-up, longer-term clinical follow-up is needed for the reliability of the marginal discoloration results of both materials.

GC is condensed and shaped by processing the surface with the help of a hand instrument following the application on the cavity with finger pressure. The consistency of GC is more liquid than the composite, making it difficult to give a natural anatomical form. However, no significant difference was observed in this study when compared with resin composite. In a clinical follow-up study by Subramaniam and others[24] using GC fissure sealant, nanoparticle content of glass carbomers was reported to increase the compressive stress and wear resistance. In contrast to this study, the GC material had a Charlie score of 8% according to anatomic form in the present study. This means that GC is not resistant to masticatory forces like resin composites.

Postoperative sensitivity, which is defined as a spontaneous or short-term pain sensation developed in response to a stimulus following the completion of restorations, is an important criterion in the evaluation of clinical studies.[25,26] Pain threshold varying by person, dentist's sensitivity, and differences in the application procedure make the evaluation of the sensitivity criterion difficult.[27] There was no statistically significant difference between the restorative materials used in terms of postoperative sensitivity (p>0.05). During the application stages of GC, no acid etching process and no additional bonding agent are required. These may be effective for the prevention of sensitivity problems.

CONCLUSION

Within the limitations of this study, the following were concluded:

1. Although similar results were obtained after 1 year of clinical follow-up for all restorative materials, a statistically significant difference was observed for marginal adaptation and marginal discoloration.
2. When the restorations made using glass carbomer filling materials were evaluated in terms of anatomic form, retention, and marginal adaptation, restorations with the Charlie score at 6 and 12 months were replaced.
3. Given the results, the glass carbomer (GC) material is recommended only as a short-term interim restoration. Further development to improve its physical properties is needed to improve the clinical performance when compared with composite resin.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of Bezmialem Vakif University. The approval code was 03/05/2017-7923.

Conflict of Interest

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

(Accepted 6 October 2021)

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Laboratory Research

Characterization of Contemporary Conventional, Bulk-fill, and Self-adhesive Resin Composite Materials

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Clinical Relevance

Bulk-fill and self-adhesive flowable resin composites are restorative materials with wide application potential in dental practice. Therefore, it is essential to know their properties, aiding in the careful decision by the dental practitioner to meet the requirements of each clinical case.

SUMMARY

Objective: To evaluate the physical and biological properties of different types of flowable resin composites and their bonding ability to dentin, comparing the performance of self-adhesive and bulk-fill materials with a conventional control.

Methods and Materials: Four flowable resin composites were tested: two self-adhesive (Y-flow [SA_YF]; and Dyad Flow [SA_DF]); one bulk-fill (Filtek Bulk Fill Flow [BF]); and one conventional composite (Opallis Flow [OF]). The microshear bond strength (μSBS) to dentin (bovine samples)

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http://doi.org/10.2341/21-063-L

was investigated at 24 hours and 6 months of storage. The materials were also characterized by degree of conversion, cross-link density, water contact angle, color stability, and cell viability (ISO 10993-5/2009) analyses. Data were analyzed using Analysis of Variance and Tukey tests (α=0.05).

Results: The μSBS values were higher for control specimens at 24 hours, whereas the resin-dentin bonds were similarly distributed among the groups after aging. Adhesive failure was the most frequent pattern observed at both time intervals. SA_YF was the only material that increased the bond strength over time. Degree of conversion increased in the following order: SA_YF (28.6±1.4%) < BF (49.7±0.8%) < OF (60.0±2.0%) = SA_DF (63.6±2.3%). Cross-link density was similar among all materials. The self-adhesive composites were more hydrophilic than the other types, with BF showing the lowest water contact angle and the greatest color alteration. All resin composites had a biocompatible behavior.

Conclusion: Chemical composition appeared to be an influential factor affecting the physico-mechanical and biological behavior of the materials tested.

INTRODUCTION

Dental resin composites are tooth-colored restorative materials with broad clinical applicability in dentistry. Despite their overall similar chemical composition (ie, a methacrylate-based organic matrix filled with silica and glass particles of varying sizes), resin composites may differ with regard to their viscous state, ranging from highly viscous materials (eg, packable composites) to those with moderate viscosity (regular composites) or low viscosity (flowable composites).[1,2] Regular resin composites have notably been the most traditional materials used in daily practice over the last few decades, while the clinical application of flowable materials has gained popularity in modern dentistry due to their versatility and applicability in several procedures. For instance, flowable resin composites are indicated as lining materials in deep cavities,[3] as restorative materials in deciduous teeth[4] or Class I/ Class V cavities of permanent teeth,[5] as sealers of pit and fissure surfaces,[6] and as the material for repairing defective resin composite[7,8] or amalgam[9] restorations.

At present, the development of flowable resin composites has shifted from manufacturing traditional materials that require prior application of adhesive systems to materials with self-adhesive properties (self-adhesive composites) or expanded polymerization depth (bulk-fill composites).[10,11] Relative to self-adhesive composites, they share the chemistry of self-etching bonding agents due to the presence of functional acidic monomers, allowing the material to create its own pathway toward the hybridization of dental substrates, without any surface pre-treatment with adhesives.[12] Equally important are the bulk-fill composites that are also associated with a simpler and less technique-sensitive restorative protocol since they can be applied in the tooth cavity with the use of thicker resin increments (eg, 4-5-mm thick) when compared with the thicknesses required when using conventional composites (<2 mm).

Although there is widespread clinical application of flowable self-adhesive/bulk-fill resin composites in modern dentistry, there is still unclear evidence regarding their overall performance. Characterization and comparative analyses of these materials could help clinicians to understand their laboratory and clinical behavior. Hence, this study aimed to evaluate the physical and biological properties of different types of flowable resin composites, and their bonding ability to dentin, with the aim of comparing the performance of self-adhesive and bulk-fill materials with that of a conventional control. The null hypothesis was that the resin composites would not differ among them, irrespective of their classification.

METHODS AND MATERIALS

Materials

Four flowable resin composites were tested in this study: two self-adhesive materials, namely Y flow SA (Yller, Pelotas, RS, Brazil, SA_YF) and Dyad Flow (Kerr Orange,CA, USA, SA_DF); one bulk-fill material (Filtek Bulk Fill Flow, 3M ESPE, St Paul, MN, USA, BF); and one conventional material (Opallis Flow, FGM, Joinville SC, Brazil, OF), serving as the control. Table 1 provides information on the manufacturer, chemical composition, color, batch number, viscous category, polymerization depth, and polymerization protocol for each resin composite according to the manufacturers' directions of use.

Study Design and Sample Size Calculation

This research followed the CRIS Guidelines for *in vitro* studies.[13] A lab assistant did the randomization and allocation of the specimens and groups. A trained operator conducted all experiments in the laboratory. The analysis and interpretation of the data were carried out by two blinded researchers who had no contact with the experimental analyses. The response variables tested in this *in vitro* study were as follows:

Table 1: *Specifications of the Resin Composites and Universal Adhesive Tested in the Study*

Material (Manufacturer)	Chemical Composition	Color	Batch Number	Flow Category	Polymerization	
					Depth	Time
Yflow SA (Yller, Pelotas, RS, Brazil)	Inorganic fillers, acid monomers (MDP and GPDM), methacrylate monomers, pigments, initiators and stabilizers	A2	3193	Low	2 mm	40 s
Dyad Flow (Kerr Orange, CA, USA)	GPDM, prepolymerized particles, barium glass fillers, colloidal silica nanoparticles, nano-sized ytterbium trifluoride fillers	A2	4910830	Low	2 mm	40 s
Filtek Bulk Fill Flow (3M ESPE, St. Paul, MN, USA)	Bis-GMA, UDMA, Bis-EMA 6, Procrylat, zirconia and silica particles, ytterbium trifluoride	U	N976617	Low	4 mm	20 s
Opallis Flow (FGM Joinville, SC, Brazil)	Silanized inorganic fillers (barium-aluminum silicate and silica nanoparticulate dioxide), methacrylic monomers, TEGDMA, Bis-EMA, Bis-GMA, camphorquinone, co-initiators, stabilizers and pigments	A2	101018	Average	2 mm	40 s
Single Bond Universal (3M ESPE, St. Paul, MN, USA)	MDP, dimethacrylate resins, HEMA, polyalkenoic acid copolymer, fillers, ethanol, water, initiators, silane	—	90424A	—	—	20 s

Abbreviations: 10-MDP, 10-methacryloxydecyl dihydrogen phosphate; GPDM, glycerol phosphate dimethacrylate; Bis-GMA, bisphenol A glycidyl methacrylate; UDMA, urethane dimethacrylate; Bis-EMA, ethoxylated bisphenol A glycidyl methacrylate; TEGDMA, tryethyleneglycol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate.

bond strength to dentin (n=20) and failure mode after bond strength testing (n=20), degree of conversion of C=C bonds (n=3), crosslink density (n=3), water contact angle (n=3), color stability (n=3), and cell viability (n=6). The primary response variable was the microshear bond strength, and the sample size calculation was based on a comparative study design of 4 independent groups, an average difference in shear bond strength of 11.5 MPa, an average standard deviation of 6.5, $\alpha = 0.05$, and test power of 0.8.[14]

Bond Strength to Dentin

Eighty bovine incisors were used, after all blood and soft tissue were removed, and thereafter stored in 0.5% chloramine/water solution at 4°C for 1 week. Then, the teeth were stored in distilled water at 4°C for no longer than two months until use. The roots of the teeth were sectioned at the cement-enamel junction, and the middle dentin was exposed by using an orthodontic grinder. The teeth were then fixed in cylindrical plastic molds with self-curing acrylic resin and the buccal surface of each tooth was ground with 180-grit SiC abrasive paper to obtain flat dentin surfaces. Subsequently, the specimens were polished with 600-grit SiC abrasive papers for 60 seconds to provide a uniform and standardized smear layer, and they were randomly allocated into four groups according to the restorative material (n=20): SA_YF, SA_DF, BF, and OF.

The resin composites were applied by using elastomeric molds with two cylindrical orifices of 1.2 mm in diameter each. In groups BF and OF, the materials were placed only after the application of a universal bonding agent (Single Bond Universal, SBU, 3M ESPE) according to the manufacturer's instructions. The bonding agent was vigorously rubbed on the dentin surface using a microbrush for 10 seconds, then the mold was placed on the adhesive layer; the bonding agent was photo-activated with a light-emitting diode (LED) curing unit (Radii Cal, SDI, Bayswater, VIC, Australia) through the orifices of the mold for 20 seconds. Next, the resin composites BF and OF were applied in the respective orifices and photo-activated with the LED for the recommended time (Table 1). In groups SA_YF and SA_DF, no bonding agent was applied so that each resin composite was applied as follows: the dentin surface was dried with absorbent paper, the elastomeric mold was placed in position, each material was inserted into the respective orifice, and photo-activation of materials was performed with the LED for the recommended time (Table 1).

All the restored samples were allocated into two subgroups, according to the period of storage in distilled

water at 37°C: 24 hours (immediate testing) or 6 months (long-term testing) (n=20). After each time interval, the microshear bond strength (μSBS) to dentin of each restored specimen was evaluated in a universal testing machine (DL500, EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 1 mm/min. To that end, the samples were mounted on the testing machine with the resin-tooth interface placed parallel to the stainless-steel wire 0.2 mm in diameter. A shear force was applied until failure. The μSBS values were calculated in MPa and considering the restoration area. Fractured specimens were observed at 40× magnification under a stereomicroscope to determine the failure mode as cohesive, adhesive, or mixed failures.

Degree of Conversion (DC)

The DC of the resin composites (n=3) was assessed using a Fourier transform infrared spectrometer (FTIR Spectrometer Prestige 21, Shimadzu, Japan) equipped with an attenuated total reflectance (ATR) device. Standardized quantities of each material were dispensed on the ATR crystal. Infrared spectra were obtained before and after photo-activation of the materials with the LED described earlier, which had an irradiance of 1000 mW/cm^2. The DC was determined by observing the ratio of the absorbance intensity of the aliphatic C=O (peak height at 1638 cm^{-1}) to the absorbance intensity of the aromatic C=C (1608 cm^{-1}) used as an internal standard.[15]

Crosslink Density

Crosslinking was indirectly measured by the percentage change in microhardness of the materials before and after storing the specimens in 100% ethanol for 24 hours (n=3). Briefly, three specimens of each resin composite (5 mm diameter × 2 mm thickness) were prepared using an elastomeric mold. The top and bottom surfaces of each specimen were photo-activated with the LED for 10 seconds. The initial (dry VHN) and final (wet VHN) Vickers Hardness Numbers of each specimen were obtained by using a microindenter (FM 700; Future-Tech Corp, Japan) with a Vickers indenter, under a load of 200 g for 15 seconds. Three indentations were performed for each specimen and the results were averaged. The ratio between ethanol-wet VHN and dry VHN (%) was used as an indication of crosslinking percentage.[16]

Water Contact Angle

The water contact angle formed on the surface of the resin composites (n=3) was measured using an Optical Tensiometer (Theta Lite TL101, Biolin Scientific Inc, Finland) with a sessile drop method. Standardized drops of distilled water (5 μL) were dispensed directly onto the surface of specimens that were made of each resin composite. The specimens were prepared as demonstrated earlier. Immediately after dispensing the drop, a dynamic reading in real time was taken of the right and left contact angles formed with the material surface. The One Attension software (Biolin Scientific Inc) was used at 20 frames per second for 20 seconds. The contact angle (°) was recorded as the mean between the right and left readings (n=3).

Color Stability (ΔE_{00})

Three cylindrical specimens (5 mm diameter × 2 mm thickness) were prepared as previously mentioned. CIELab color parameters were measured with a VITA Easyshade Spectrophotometer (Zahnfabrik, BadSäckingen, Germany) against white (L*=93.1, a*=1.3, b*=5.3) and black (L*=27.9, a*=0, b*=0) backgrounds. The measurements were made immediately after polymerization (first reading) and after storing the specimens in distilled water at 37°C for 6 months (second reading). The color alteration (ΔE_{00}) of the resin composites after storage was calculated based on the CIEDE2000 equation:[17]

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

where, ΔL', ΔC', and ΔH' are the differences in brightness, chroma, and hue between two sets of color coordinates; RT is the rotation function that explains the interaction between differences in chroma and hue in the blue region; SL, SC, and SH indicate the weighting functions used to adjust the total color difference to variation in perceived magnitude, with variation in the location of difference in the color coordinate between two color readings; and KL, KC, and KH represented the correction terms for the experimental condition. The perceptibility threshold was established at $\Delta E_{00} = 0.8$, whereas the acceptability threshold was set at $\Delta E_{00} = 1.8$.[18]

Cell Viability

The cytotoxicity test was performed according to ISO 10993-5: 2009,[19] using mice fibroblast cells (L929) cultured at a density of 2×10^4 cells in 96-well plates containing DMEM (Eagle's Medium Modified by Dulbecco) supplemented with 10% L-glutamine, 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 U/mL). The cells were incubated at 37°C in 95% air and 5% CO_2 for 24 hours, and the cell viability ratio was evaluated by the MTT assay. Cylindrical shaped specimens were prepared (5×1 mm,

n=3). The specimens were placed in 24-well plates with 1 mL DMEM and stored at 37°C and pH 7.2 under static conditions. After 24 or 48 hours, 200 μL of each specimen was transferred to the 96-well plate containing the precultured cells, and the plate was incubated for another 24 hours. As a control, a group containing only fibroblast cells in DMEM was used. After incubation, DMEM was removed and an MTT solution was placed in each well. After 4 hours of incubation at 37°C in a condition of darkness, the blue formazan precipitate was extracted from the mitochondria using 200 μL/well of dimethyl sulfoxide (DMSO) on a shaker at 150 rpm for 5 minutes. The absorption was determined using a spectrophotometer at a wavelength of 540 nm.

Statistical Analysis

For statistical analysis, the statistical method was based on adherence to the normal distribution model and equality of variances. For the microshear bond strength, a one-way analysis of variance (ANOVA) was used to detect significant differences between the materials for each storage period. The effect of aging condition was analyzed using a two-sample Student's-*t* test. For the properties of crosslink density, degree of conversion, and color stability, one-way ANOVA was used. Two-way ANOVA analysis was used to evaluate the effect of the material and the incubation time on cell viability. All the multiple comparisons were performed using the Tukey *post-hoc* test. For all tests, $p<0.05$ was considered statistically significant. The statistical analyses were carried out using SigmaPlot 12 software (Systat Inc, San Jose, CA, USA).

RESULTS

The results for the bond strength to dentin and fracture pattern of the resin-dentin bonds are shown in Figure 1. At immediate testing (24 hours of water storage), the control group (OF) demonstrated higher bonding performance than the other groups ($p\leq0.003$), which did not differ among them ($p\geq0.214$). In the long-term test (6 months of water storage), all groups performed in a similar manner to each other ($p\geq0.181$). When the immediate μSBS values of the same resin composite group were compared to the long-term values, the resin-dentin bonds were stable over time for SA_DF, BF, and OF, whereas for SA_YF the μSBS values were statistically increased after 6 months of water storage ($p=0.03$). Regarding the failure pattern obtained for each group in the two storage time intervals, all groups demonstrated a predominance of adhesive failures, with the control (OF) showing nearly 20% of mixed fracture and the BF and SA_DF groups exhibiting approximately 30% of pre-test failures after 6 months of water storage (Figure 1B).

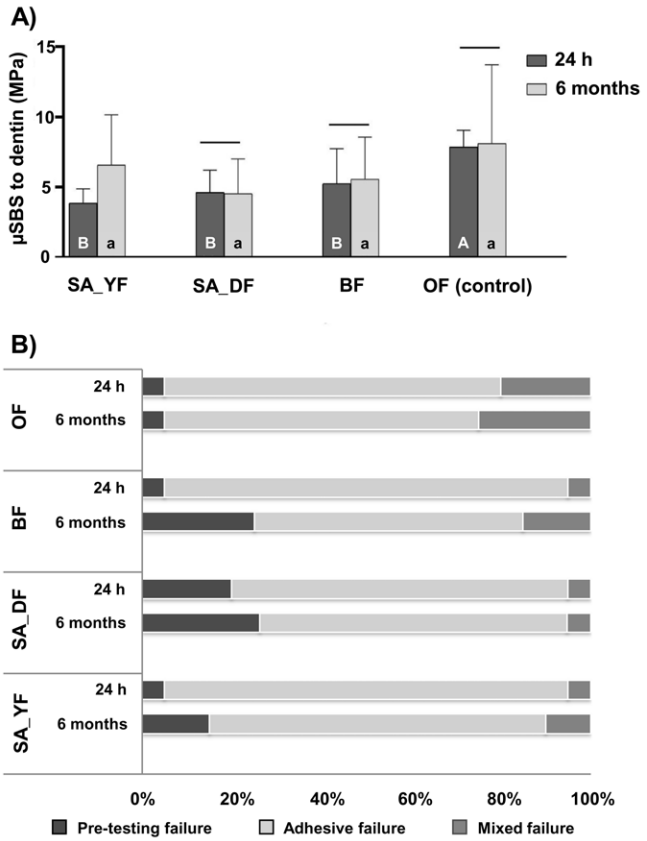

Figure 1. *(A): Graphs showing microshear bond strength to dentin. The same uppercase and lowercase letters indicate no statistically significant differences between resin composites at 24 hours and 6-month time intervals, respectively (p<0.05). Bars under the same horizontal line indicate statistically similar bond strength values when immediate and long-term time intervals were compared (p>0.05). (B): Failure mode of resin composites tested at immediate (24 hours) or long-term (6 months) time intervals. Abbreviations: SA_YF, Y-flow Self-Adhesive; SA_DF, Dyad Flow Self-Adhesive; BF, Filtek Bulk Fill Flow; OF, Opallis Flow.*

Table 2 shows the results for the degree of conversion, crosslink density, and water contact angle of the resin composites tested in the study. Relative to the degree of conversion, SA_YF had the lowest conversion of C=C double bonds, followed by BF. These resin composites resulted in lower polymerization levels than the SA_DF and control materials ($p<0.001$), which did not differ between each other ($p=0.129$). All materials showed statistically similar crosslink density values ($p=0.620$). BF demonstrated the least hydrophilic behavior of tested materials, followed by the control, which all resulted in higher water contact angle values when compared with the self-adhesive materials ($p\leq0.001$); SA_YF and SA_DF did not differ between each other ($p=0.202$).

Table 2: *Results (Mean and Standard Deviation) for the Cross-Link Density (CLD), Degree of Conversion (DC), and Water Contact Angle (WCA) of Groups Tested*[a]

Group	DC (%)	CLD (%)	WCA (°)
SA_YF	28.6 (1.4) C	83.1 (2.2) A	60.9 (2.3) C
SA_DF	63.6 (2.3) A	89.7 (9.8) A	57.3 (3.4) C
BF	49.7 (0.8) B	82.9 (9.0) A	70.7 (2.0) A
OF (control)	60.0 (2.0) A	86.3 (5.3) A	65.0 (2.3) B

Abbreviations: SA_YF, Y-flow Self-Adhesive; SA_DF, Dyad Flow Self-Adhesive; BF, Filtek Bulk Fill Flow; OF, Opallis Flow.
[a]*Mean (standard deviation); distinct uppercase letters in the same column indicate statistically significant differences among the groups (*p*<0.05).*

The results for the color alteration of materials after water storage for 6 months are shown in Figure 2A. BF demonstrated higher ΔE_{00} values than the other resin composites ($p \leq 0.001$), which did not differ among each other ($p \geq 0.677$). The control (OF) was the only resin composite that resulted in color alteration values below the 50% acceptability threshold (ΔE_{00}=1.8). Lastly, the results for the cell viability assay are presented in Figure 2B. According to the statistical analysis, cell viability was significantly influenced by both material and incubation time ($p<0.001$), and a significant interaction between these two variables was also observed ($p<0.0001$). At the 24-hour time interval, cell viability was statistically similar among the different resin composites ($p>0.05$), whereas it increased in the following order at the 48-hour time interval: SA_YF < SA_DF < OF (control) = BF. While the BF and OF materials displayed similar biocompatibility at both time intervals investigated, cell viability was significantly reduced at 48 hours for the self-adhesive resin composites.

DISCUSSION

The present study compared the effects of three contemporary resin composites with low viscosity to that of a flowable conventional restorative material.

Overall, the materials tested in this study performed adequately in terms of physical and biological properties. However, our main goal was to investigate the bonding ability to dentin of the different types of flowable resin composites, since this analysis helps to predict their clinical performance.[20] At the immediate test, the two self-adhesive materials resulted in resin-dentin bonds similar to those of the bulk-fill material (Figure 1A), in spite of their different degree of conversion values. Here, we may assume that the similar bonding mechanism of all the three foregoing resin composites had an influence on their similar bonding ability. Indeed, SA_YF and SA_DF are composed of GPDM and 10-MDP monomers, which create the necessary acidic environment for etching dentin; the flowable nature of the materials may also allow resin infiltration into the etched dentin, creating an adequate hybrid layer. The other resin composites had a similar bonding mechanism, in which both the bulk-fill and the conventional control were applied after application of a universal bonding agent based on 10-MDP. It is of the utmost importance to understand that the self-adhesive resin composites created resin-dentin bonds

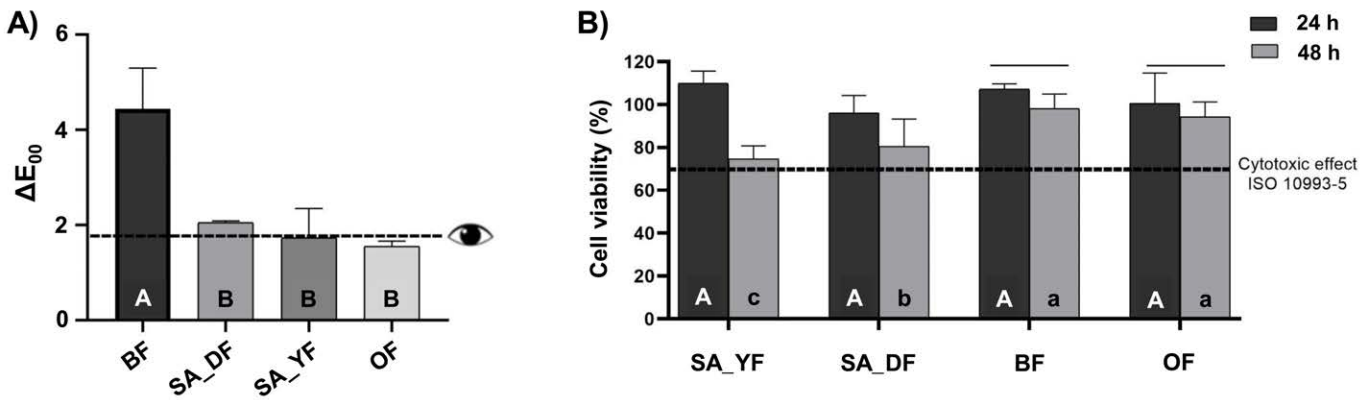

Figure 2. *(A): Graphs showing color alteration results (ΔE00). For color alteration, the same uppercase and lowercase letters indicate no statistically significant differences between resin composites at 24 hours and 6-month time intervals among resin composites (*p*<0.05); value indicated by dotted line at ΔE = 1.8 corresponds to 50% visual acceptability under clinical conditions, which was used as a threshold for visually detectable color changes. (B): Cell viability results. For cell viability, the same uppercase and lowercase letters indicate no statistically significant differences among resin composites at 24-hour and 48-hour time intervals, respectively (*p*<0.05). Bars under the same horizontal line indicate statistically similar cell viability between different incubation time intervals (*p*>0.05). Abbreviations: SA_YF, Y-flow Self-Adhesive; SA_DF, Dyad Flow Self-Adhesive; BF, Filtek Bulk Fill Flow; OF, Opallis Flow.*

that were statistically similar to the bonds derived from the bulk-fill material, confirming the bonding ability of the former. Nevertheless, the conventional control resulted in stronger resin-dentin bonds, probably due to the considerably high conversion of monomers of this group in particular.

Notably, the higher the level of polymerization of the restorative material, the better its cohesiveness and mechanical strength during fracture. A higher number of mixed failures also occurred in the control, which suggested a more densely packed structure that had a positive influence on the bond strength results. More importantly, OF seemed to contain a larger amount of filler particles, since it is a material with average flowability, differing from the other materials that have a less viscous consistency (low viscosity category). Here, the higher the level of filler loading, the lower the negative effects of polymerization stress,[21] and consequently, the better the quality of resin-dentin interfaces. This would perhaps explain the better bonding performance of the control over the self-adhesive and bulk-fill resin composites in the immediate test time interval.[22]

It is known that the degree of conversion of resin-based polymer systems may increase after *in situ* polymerization,[23] therefore the resin-dentin bonds may also become stronger with time.[24] This was especially true for SA_YF, which showed increased bond strength values after long-term water storage, possibly due to the gain in the conversion of monomers, which were previously low (<30%) at immediate testing, strengthening the hybrid layer during aging simulation. Moreover, SA_YF is a HEMA-free material, and considering that HEMA can inhibit the adhesion mechanism of acidic monomers,[25,26] one may suggest that hybridization with SA_YF formed a stable adhesive interface with strengthening potential. An aspect that deserves further discussion relates to the presence of 10-MDP in the resin-dentin bonds created with the application of SA_YF, BF, and OF materials, but not with application of SA_DF. The 10-MDP monomer is one of the most important acidic ingredients responsible for the formation of stable hybrid layers over time,[27] since it is capable of creating a stable 10-MDP-calcium salt with low solubility, thus forming a nano-layered bonding interface between the resin phase and hydroxyapatite.[28] Remarkably, this nano-layer has greater resistance to hydrolysis, as suggested elsewhere.[29]

The degree of conversion varied widely among the resin composites, with the two self-adhesive materials exhibiting the lowest (SA_YF) and the highest (SA_DF) polymerization states of the materials tested in the study (Table 2). Remarkably, the conversion of monomers is an intrinsic property relying on chemical features such as the type of resin monomers, type/concentration of initiation system, and rheological characteristics of materials.[30] Bearing this in mind, something related to the chemistry of SA_YF might have played a role in the ~2.2 times lower DC values, when compared with the SA_DF counterpart. Here, both self-adhesive composites were composed of functional acidic monomers, which are usually characterized by having only one polymerizable methacrylate group per molecule, thus limiting the material to acquiring an extended level of polymerization.[31] However, one may understand that SA_YF and SA_DF are both composed of GPDM (Table 1), which is an acidic monomer that has two polymerizable methacrylate groups, ie, a factor that may have positively influenced polymerization of the materials.[32] Conversely, while SA_DF resulted in high conversion of monomers, SA_YF yielded values below the 30% level. We may assume that the presence of 10-MDP, which is an acidic monomer with only one methacrylate group available for polymerization, contributed to the reduced polymerization level of SA_YF.

Despite the differences in polymerization levels reached by each resin composite, the materials did not differ in terms of their crosslink density. Overall, this property relates to the amount of chemical reactions that link various polymer chains together, playing an important role in the physico-mechanical behavior of resin-based polymer systems.[33] The method used in our study to determine the material crosslink density was to evaluate the percentage reduction in hardness after immersion in ethanol, which provided an indirect estimation only. Nevertheless, it may add to understanding of the crosslink state of the materials tested.[34,35] It is noteworthy that the crosslink density of the contemporary resin composites was similar to that of the control, suggesting that all materials shared a similar stability when exposed to a hydrolytic medium. Thus, we may assume the materials may undergo hygroscopic and hydrolytic degradation in the same manner, which is interesting for confirming the clinical suitability of the self-adhesive and bulk-fill materials when compared with the conventional control.[33]

The characteristic of wettability was also investigated in our study, since this surface property may be positively correlated to the bonding ability of resin composites.[36] Although the materials displayed water contact angle values lower than 90°, suggesting their hydrophilic behavior,[37] the bulk-fill composite was the least hydrophilic of the materials tested in the study, followed by the control and then by the self-adhesive materials (Table 2). Of note, the presence of functional

acidic monomers (eg, GPDM or 10-MDP) in the self-adhesive resin composites may have increased their hydrophilicity, since both acidic monomers have a hydrophilic moiety (eg, pendant hydroxyls) capable of increasing wettability properties.[32] Despite the similar water contact values obtained when comparing the two self-adhesive composites, SA_DF showed the lowest values, probably due to the more hydrophilic nature of GPDM when compared with 10-MDP.[31,32] Indeed, considering that SA_YF is composed of both GPDM and 10-MDP, one may infer that hydrophilicity would be slightly lower in the aforementioned resin system.[7] Despite the importance of hydrophilicity for adhesion purposes, the higher the hydrophilic behavior of a resin system, the more hydrolytically unstable it will be.[38] Here, we did not evaluate the hygroscopic and hydrolytic properties of the resin composites which, therefore, deserves further investigation. However, we may assume that the bulk-fill resin composite would demonstrate a slightly greater resistance to hydrolysis due to its higher water contact angle values. This may be explained by the procrylat-based composition of BF (Table 1) since this ingredient is a proprietary monomer analog to Bis-GMA, showing an extended molar mass but still having properties of low viscosity and low water solubility, thereby contributing to a less hydrophilic polymer system, as verified in our findings.

The present study also characterized the resin composites by evaluating their color stability after long-term (6 months) water storage. Color plays an important role in obtaining optimal esthetics in resin composite restorations, and color stability may depend on several compositional features, such as the type of resin matrix, the size and shape of filler particles, the depth of polymerization achieved by the material, and the nature of pigments present for making the restorative tooth-colored.[39] As shown in Figure 2A, the control group (OF) was the only resin composite that resulted in $\Delta E_{00} \leq 1.8$ (ie, below the 50% acceptability threshold),[18] thereby representing the most physically stable material. One may suggest that silane functionalization of the inorganic fillers found in OF created a strong chemical interaction between resin matrix and filler phase, resulting in a material with greater color stability.[40] Considering that information on the silanization of fillers for the other resin composites was not supplied (Table 1), we may assume these materials would undergo faster discoloration than the control. One interesting finding of our study was that the bulk-fill resin composite exhibited the greatest color alteration, which was at least two times higher than that of the other materials. Indeed, earlier in the article we inferred that the bulk-fill material would offer greater resistance to hydrolysis due to its more hydrophobic behavior, but according to the color alteration results, BF suffered from color instability to a greater extent, suggesting an extended degradation profile compared to the other materials. Notably, aspects such as the universal color shade of BF and its characteristic of increased depth of polymerization (ie, 4 mm), which differed from the other resin composites, could have played a role in the overall absorbance and reflection of light within the bulk-fill composite, making the material optically less stable after long-term wet storage.[41] Equally as important, the self-adhesive composites showed ΔE_{00} values close to the 1.8 threshold and statistically similar to the control, indicating their suitability as restorative materials, especially for creating restorations with durable esthetics.[18]

With regard to cell viability, all resin composites demonstrated a non-toxic behavior, resulting in cell proliferation levels above 70% (Figure 2B), thus reaching the requirement for a biocompatible material.[19] It is important to highlight that resin composites are expected to release uncured monomers, oligomers, or other non-reacted ingredients (eg, initiators, stabilizers, pigments) after polymerization, posing a risk for cell toxicity.[42-44] Notwithstanding, cell viability was similarly distributed among the resin composites at the 24-hour time interval, indicating their clinical safety at earlier stages. One may consider this result an advantage for the self-adhesive composites, since they represent the most recent advancements in the development of resin-based restorative materials, and due to their biocompatible properties, dental practitioners could more readily begin to consider the clinical use of these restoratives. However, SA_YF and SA_DF created a considerably lower cell viability scenario at the 48-hour time interval when compared with the bulk-fill and control materials, probably due to the more acidic behavior of the former.[45] It is important to highlight that despite the reduction in cell viability, the self-adhesive composites have still performed as non-toxic materials, and the reduction in their biocompatibility may be a consequence of the release of unreacted acidic monomers, which may result in an initial acidic medium, although without an intense cytotoxic potential.[46] It is worth mentioning that the polymerization of dimethacrylate-based resins is never complete, so that some cytotoxic reaction may occur over time due to the release of unpolymerized monomers.[43]

The present study evaluated the mechanical, physical, and biological properties of different contemporary flowable resin composites, and according to our findings, the restorative materials performed mostly

different among them, thus leading to the rejection of the null hypothesis.

CONCLUSIONS

Within the limitations of this *in vitro* study, it could be concluded that flowable resin composites of different categories, ie, self-adhesive, bulk-fill, or conventional materials, performed differently among each other, depending on the properties investigated. The chemical composition of materials appeared to be an influential factor on their physico-mechanical and biological behavior. Overall, the self-adhesive flowable resin composites showed promising results.

Acknowledgments

This study was supported by CAPES - Finance Code 001 and Foundation for Research Support of the State of Rio Grande do Sul: FAPERGS, Grant # 19/2551-0001639 and # 21/2551-00006.

Conflict of Interest

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

(Accepted 6 August 2021)

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Influence of the Flowable Resin Layer on Bond Strength Between Resin Cement and a Universal Adhesive Applied in the Immediate Dentin-sealing Technique

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Clinical Relevance

The immediate dentin-sealing technique is a promising method for the optimization of adhesive procedures in indirect restorations. This clinical protocol benefits from the use of universal adhesive systems associated with a low-viscosity resin layer. Reduction of postoperative sensitivity and better bond strength values are relevant clinical contributions of the technique.

SUMMARY

Objectives: The present study evaluated the influence of a flowable resin layer on bond strength between resin cement and a universal adhesive applied using an immediate dentin sealing (IDS) technique.

Methods and Materials: Coronary portions of bovine teeth were randomly divided into six groups (n=15). In the IDS.U group, the exposed dentin was immediately sealed with the Single Bond Universal adhesive (3M ESPE) following the self-etching protocol. In the IDS.UF group, a layer of Filtek Z350 (3M ESPE) flow resin was applied over the universal adhesive. In the DDS (control) group, the dentin was kept "fresh" and delayed dentin sealing was performed. After 24 hours in distilled water at 37°C, dentin surfaces were treated

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http://doi.org/10.2341/21-050-L

with pumice, phosphoric acid, and the application of the universal adhesive in the IDS.U and IDS. UF groups. The DDS group was treated with pumice and the universal adhesive was applied. The samples received cylinders of resin cement Rely X Ultimate (3M ESPE) made with the aid of starch tubes of 0.96 mm in diameter and 2 mm in length. They were submitted to the microshear bond strength test (μSBS) at 0.5 mm/min, after 24 hours (T1) and 3 months (T2). The fracture areas were evaluated qualitatively using a DSM 300 microscope (KOZO) with 45× magnification and classified as: adhesive, cohesive in cement, cohesive in dentin, or mixed. Samples were analyzed by scanning electron microscopy (SEM). The data were compared statistically between groups using the Kruskal-Wallis test, and intra-groups using the Mann-Whitney test (α=0.05).

Results: There were no significant differences between groups for the bond strength values (p>0.05). The IDS.UF group showed higher values at 3 months, when compared to the values of 24 hours (p<0.001). All groups showed a predominance of adhesive fracture (86.7% to 100%). SEM showed dentinal tubules exposed in the IDS.U and DDS groups; in the IDS.UF group, the tubules were completely sealed.

Conclusions: The flow resin can be used on the adhesive when using the IDS technique because it increased the bond strength values after 3 months and promoted effective sealing of the dentinal tubules.

INTRODUCTION

Dental preparation for indirect restorations often exposes dentin. The exposed dentinal tubules allow the transmission of mechanical and chemical stimuli to the dental pulp, which can cause sensitivity and even irreversible pulp damage.[1] In the conventional protocol for indirect restorations, the exposed dentin is sealed only at the time of cementation, being subject to contamination throughout the period of provisional restoration.[2]

Immediate dentin sealing (IDS) is an alternative method to the conventional protocol, in which the exposed dentin is sealed with an adhesive system immediately after preparation and prior to the execution of the impression.[3] Thus, dentin and, consequently, dentinal tubules are sealed, providing protection against bacterial contamination and the action of impression materials. The IDS also makes it possible to reduce dentin sensitivity.[3] It is known that "fresh" dentin, freshly prepared, is the ideal substrate for adhesion, as it is less likely to be contaminated, resulting in greater final bond strength.[3] In addition, when IDS is performed, higher values of adhesive strength of resin cement to dentin are obtained when compared to delayed dentin sealing (DDS).[4]

There are several clinical protocols, associated with different materials, that can be used to perform the IDS technique, varying the dentin hybridization from adhesive systems of total acid conditioning (etch-and-rinse),[2-9] self-etch systems,[4,5,10-13] and techniques that apply a layer of flow resin over the previously hybridized substrate.[4,11,13-19] According to Nikaido and others,[14] the application of flow resin on the dental adhesive, in addition to helping to protect the dental pulp, also promotes the greater reduction of dentinal sensitivity at the time of insertion and removal of provisional restorations, which demonstrates a notable clinical advantage.

Recently, IDS has been performed with universal adhesives,[11,13,17] also contributing to higher values of dentin bond strength when compared to the delayed dentin sealing protocol. The use of universal adhesives in IDS can make the technique less susceptible to errors, considering the possibility of applying the adhesive in a single step.[11,13,17] Therefore, the use of universal adhesives reaffirms the advantages generated by not using acid conditioner on the dentin surface initially presented in self-etching systems.[20] Among the main clinical advantages, there is less postoperative sensitivity and also greater bond stability, when compared to the hydrolytic degradation of collagen observed in etch-and-rinse systems.[21] The option for "multi-modal" adhesives thereby is also a relevant alternative in the application of the IDS technique for immediate protection of the dentin substrate.

Based on the scientific evidence that supports the immediate sealing of dentin, as well as the positive results associated with the use of a layer of flow resin on the dental adhesive in IDS, studies that evaluate the applicability of new generations of adhesives and resin cements in this technique are fundamental. Hence, studies are important to contribute to the definition of satisfactory and safe clinical protocols for the performance of the IDS in the cementation of indirect restorations on sealed dentin. The present *in vitro* study evaluated the influence of the flowable resin layer on bond strength between resin cement and a universal adhesive used in an IDS technique. The tested hypotheses were that the flow on the universal adhesive does not interfere with the bond strength results of the

resin cement and improves the quality of the dentin seal when used in an IDS technique.

METHODS AND MATERIALS

Tooth Preparation

Bovine lower incisors were used, which were cleaned and packed in neutral 0.1% thymol solution at 6ºC to prevent dehydration and bacterial proliferation. The root portions of the teeth were removed with the aid of a double-sided diamond disc (KG Sorensen, São Paulo, SP, Brazil). The vestibular face of the coronary portion was inserted about 1 mm in depth in a utility wax slide (Lysanda, São Paulo, SP, Brazil).

A wooden matrix with 20-mm diameter perforations was placed over the wax, surrounding the coronary portion of the teeth. Epoxy resin (Cristal, Redelease, São Paulo, SP, Brazil) was carefully manipulated and poured in during the sandy phase until it filled the entire wood matrix perforation. After about 4 hours, the time required for resin setting, the epoxy resin cylinder and tooth assembly were removed from the matrix.

The enamel of the labial face of the teeth was removed using a PVV polisher (Teclago, Vargem Grande Paulista, SP, Brazil) associated with silicon carbide sandpaper #100 (Norton, Guarulhos, SP, Brazil) to expose the dentin. Then, for flattening the surface, sandpapers #320 for 10 seconds and #600 for another 10 seconds were used.

The dentin surfaces were cleaned using a pumice paste (Polidental Ind Com Ltda, Cotia, SP, Brazil) manually prepared into a dappen glass with a small amount of water. The pumice was then applied with a rotary brush for 15 seconds, followed by abundant washing with air-water spray for 15 seconds.

Dentin Surface Treatments

Table 1 lists the trade names, manufacturers, and compositions of the main materials used in the study.

The prepared teeth were randomly divided into six groups (n=15). In the IDS.U group, the exposed dentin was immediately sealed with Single Bond Universal adhesive (3M ESPE), following the self-etching protocol. The adhesive was actively applied with microbrush and friction for 20 seconds, followed by the application of a light air jet for 5 seconds, as recommended by the manufacturer, and light curing for 20 seconds at 1000 mW/cm^2 (Emitter D, Schuster, Santa Maria, RS, Brazil).

Table 1: *Materials Used in the Study*

Commercial Name	Manufacturer	Main Components
RelyX Ultimate	3M ESPE, St Paul, MN, USA	Base paste: Methacrylate monomers, radiopacifiers, silanized filler particles, initiator components, stabilizers, rheology additives Catalyst paste: Methacrylate monomers, radiopacifiers, alkaline filler particles, stabilizers, pigments, rheology additives, fluorescent components, light-free polymerization activator for Single Bond Universal
Single Bond Universal	3M ESPE, St Paul, MN, USA	Alcohol, water, silicon-treated silica, 10-MDP, Bis-GMA, 2 hydroxyethyl methacrylate, decamethylene dimethacrylate, acrylic copolymer and itaconic acid, camphorquinone, N, N-dimethylamonoethyl, methyl ketone, silane
Filtek Z350 XT Flow Resin	3M ESPE, St Paul, MN, USA	Bis-GMA, UDMA, TEGDMA, PEGDMA, Bis-EMA, ytterbium trifluoride, silica particles, zirconia
Pumice	Polidental Ind Com Ltda, Cotia, SP, Brazil	Quartz
Alpha Etch Gel	Nova DFL, Rio de Janeiro, RJ, Brazil	37% Phosphoric Acid

Abbreviations: MDP, methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol A-glycidyl methacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; PEGDMA, polyethylene glycol dimethacrylate; Bis-EMA, bisphenol A ethoxylated dimethacrylate.

In the IDS.UF group, to cover the universal light-cured adhesive, a thin layer of Filtek Z350 flow resin (3M ESPE) was applied, followed by light curing for 20 seconds (1000 mW/cm^2). In the DDS (control) group, the dentin was kept "fresh."

The teeth were stored in distilled water and placed in an oven at 37°C for 24 hours.

Resin Cement Cylinders

After the 24-hour storage time, the sealed dentin surfaces were cleaned with pumice, as previously described. In the IDS.U and IDS.UF groups, 37% phosphoric acid was applied for 30 seconds, followed by a rinse with an air-water spray for 30 seconds, and air-jet drying for 5 seconds. A new application of the universal adhesive was performed with a microbrush and friction for 20 seconds, followed by the application of a light air jet for 5 seconds to evaporate the solvent, as recommended by the manufacturer, and light cured for 20 seconds at 1000 mW/cm^2 (Emitter D, Schuster, Santa Maria, RS, Brazil). In the DDS group, delayed dentin sealing was performed with a universal adhesive, following the self-etching protocol, as previously described for the IDS.

A double-sided adhesive tape with four circular perforations made with a rubber dike perforator (hole n° 5) was fixed over the sealed dentin surfaces. Starch tubes (Renata, Pastificio Selmi, Londrina, PR, Brazil) were fixed on the areas delimited by the perforations (0.96 mm in internal diameter and 2 mm in height).[22] The starch tubes were filled with Rely X Ultimate resin cement (3M ESPE) followed by light curing for 40 seconds at 1000 mW/cm^2 (Emitter D).

The teeth with polymerized resin cement cylinders inside the starch tubes were stored in closed plastic containers, submerged in distilled water, and placed in an oven at 37°C. Fifteen specimens from each group were stored for 24 hours (T1), and another 15 specimens from each group were stored for 3 months (T2). After times T1 and T2, the starch tubes were carefully removed with the aid of a scalpel blade number 15 (LAMEDID, Osasco, SP, Brazil). The resin cement cylinders then proceeded to the test of micro-shear bond strength. Figure 1 shows the study flowchart.

Micro-shear Bond Strength Test

After the storage times T1 and T2, each cylinder of resin cement was subjected to the micro-shear bond strength test. An active tip in the form of a knife blade was applied in the region of the bond area, parallel to the dental surface, with a speed of 0.5 mm/min, in the Instron 5965 universal testing machine. The values recorded in newtons (N) were divided by adhesion area, to calculate the bond strength (MPa).

Failure Analysis

The cylinder fracture surface areas were qualitatively assessed using a DSM 300 microscope (KOZO) with 45× magnification and classified as: adhesive—failure in the adhesion interface; cement cohesive—cement failure; dentin cohesive—dentin failure; or mixed.

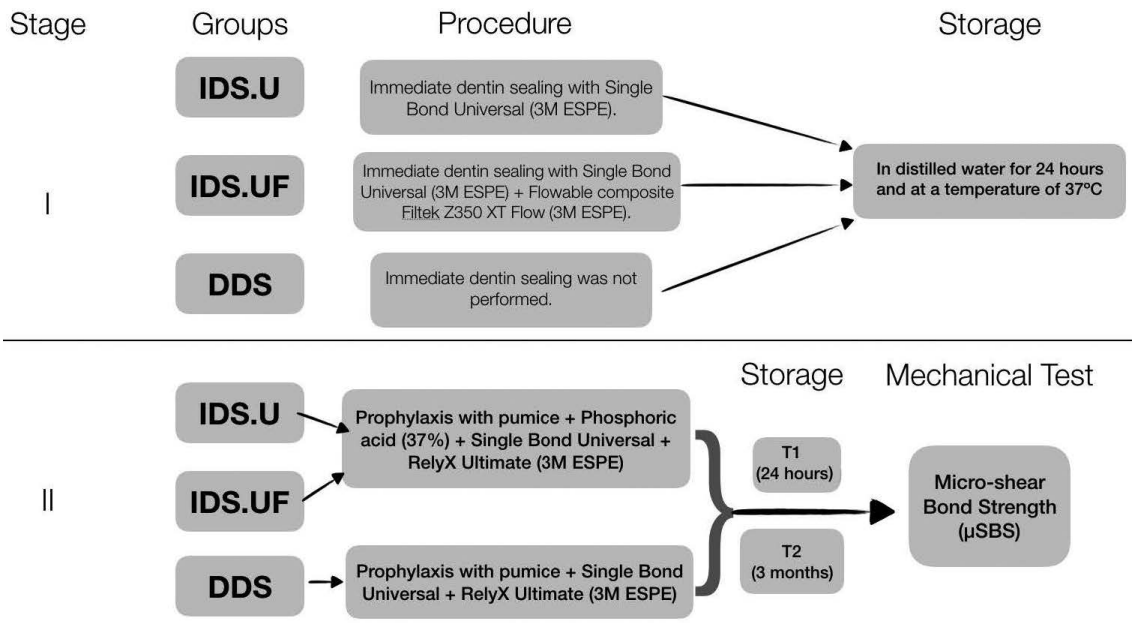

Figure 1. *Study flowchart (n = 15).*

Scanning Electron Microscopy (SEM)

Two samples from each group were analyzed using a scanning electron microscope JEOL JSM-IT300 (JEOL Brasil Inst Científicos Ltda, Sao Paulo, SP, Brazil) with 5000× magnification. For the IDS.U and IDS.UF groups, a sample was separated after the IDS protocol and another after treating the sealed surface with pumice, phosphoric acid, and universal adhesive. For the DDS group (control), a sample of "fresh" dentin was separated; and another after the delayed dentin sealing with universal adhesive.

Statistical Analysis

The data were submitted to statistical analysis using the SPSS 24 software (SPSS Inc, Chicago, IL, USA) at a significance level of 5%. The values of the micro-shear bond strength (MPa) were assessed for homogeneity of variances by the Levene test, and for normal distribution using the Shapiro-Wilk test. Due to the absence of normality and non-homogeneous variances, the bond strength data were compared between groups using the Kruskal-Wallis test. For intra-group comparisons of data obtained at 24 hours and 3 months, the Mann-Whitney test was used. Descriptive analysis of the frequencies observed for the fracture pattern and the SEM images was performed.

RESULTS

The bond strength values obtained in the studied groups (Table 2) were statistically similar to each other, both at 24 hours (p=0.107) and at 3 months (p=0.074). In intra-group comparisons, significant statistical differences were found only for the IDS.UF group (p<0.001), with higher values in 3 months. The IDS.U groups (p=0.526) and the DDS group (p=0.185) did not show significant differences between the times of 24 hours and 3 months. Table 2 presents descriptive statistics, with graphical representation in the Box-plot (Figure 2).

Table 2: *Median, Inter-Quartile Interval (Q25-Q75) and Minimum and Maximum Values (Min-Max) for the Data of the Micro-Shear Bond Strength (MPa) in the Different Groups*

Groups	Storage	Median	Q25-Q75	Min-Max
IDS.U	24 hours	3.71	2.61-7.54	1.83-10.62
	3 months	4.62	2.77-8.71	1.72-13.91
IDS.UF*	24 hours	2.76	2.01-3.75	1.57-4.61
	3 months	5.49	4.01-8.79	2.79-14.34
DDS	24 hours	3.35	2.45-4.25	1.57-5.69
	3 months	4.10	3.19-4.63	1.94-6.27

**Significant statistical differences between times (p<0.001, Mann-Whitney test)*

According to the qualitative analysis of the fracture pattern, all groups presented predominantly adhesive-type fractures, as shown in Table 3.

As for the analysis of scanning electron microscopy,the DDS group presented images of "fresh" dentin with the presence of smear layer, occluded dentinal tubules, and irregular surface (Figure 3A); it was possible to observe the exposure of dentinal tubules after prophylaxis and application of the adhesive (Figure 3B). For the IDS.U group, a partially sealed dentin surface was observed (Figure 3C), and the presence of exposed dentinal tubules after treatment of the sealed dentin with prophylaxis, acid conditioning and application of the adhesive (Figure 3D). The IDS.UF group had a smoother and more regular surface than the other groups (Figure 3E), and no exposed dentinal tubules were observed after performing the treatment of sealed dentin with prophylaxis, acid conditioning and application of the adhesive (Figure 3F).

DISCUSSION

The present work evaluated the influence of the application of flow resin on the micro-shear bond strength of resin cement to dentin immediately sealed with a universal adhesive system. The *in vitro* evaluation of this technical IDS emerges as an important precursor to clinical studies, given the relevant benefits generated by the mechanism of action of "multi-modal" adhesives, which can be used without acid etching in dentin, in

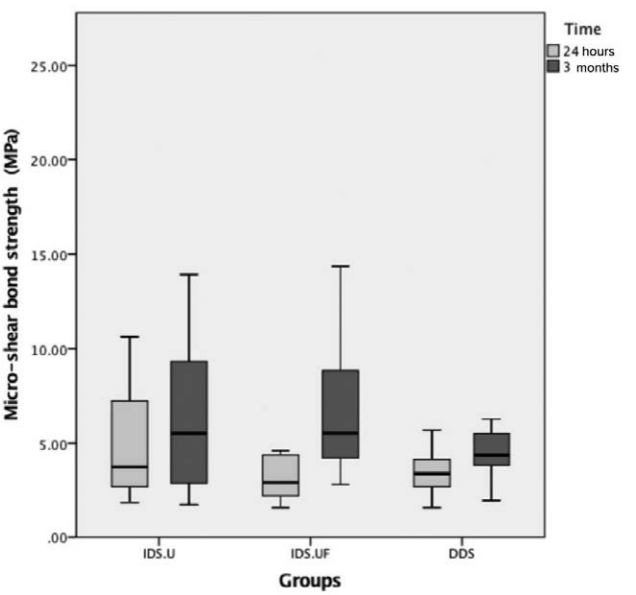

Figure 2. *Box-plot of the values of the micro-shear bond strength (MPa) for the different groups at 24 hours and 3 months.*

Table 3: *Absolute Frequencies (Percentages) for the Fracture Pattern in the Different Groups*

Groups	Storage	Fracture Pattern	
		Adhesive	Mixed
IDS.U	24 hours	14 (93.3%)	1 (6.7%)
	3 months	15 (100%)	0 (0%)
IDS.UF	24 hours	15 (100%)	0 (0%)
	3 months	15 (100%)	0 (0%)
DDS	24 hours	13 (86.7%)	2 (13.3%)
	3 months	15 (100%)	0 (0%)

addition to establishing a chemical interaction with this substrate, due to the incorporation of the acidic molecule 10-MDP.[23] These characteristics are also desirable for obtaining and maintaining a resistant protective layer on the dentin surface that in the future will interact with the cementing agent and the indirect restoration.

Furthermore, the results of this research corroborate with the philosophy that dentin should be sealed immediately and that this seal does not impair the bond strength when using a dual resin cement.[4,13,24] Regarding the use of a flow resin layer over the adhesive, the studies by Knobloch and others[24] and Van den Breemer and others[4] found that the low viscosity resin did not result in increased bond strength for the tested protocols. Nevertheless, other studies[11,19,31,32] found that the use of flow resin increased the bond strength of resin cement to dentin subjected to immediate sealing. In the present work, the application of flow resin did not promote a significant increase in bond strength when compared to the other groups. Therefore, the hypothesis that flow resin does not interfere with the bond strength values of resin cement to sealed dentin was accepted.

Another important aspect refers to the sample aging method carried out in the study, which possibly influenced the non-significant statistical differences in bond strength between the groups tested. The contact with elastomeric impression materials, the use of provisional restorative materials, and the placement of the samples in artificial saliva are procedures that would contribute to better adhesive performance of the immediate sealed groups. In this case, the specimens submitted to the delayed dentin sealing (DDS) would be more affected by this aging method as a result of nonprotection of the exposed dentin. This fact was confirmed in another study[33] and brings a solid explanation for the outcome found in the present study.

Additionally, in intra-group comparisons, the bond strength values obtained after 3 months of storage were

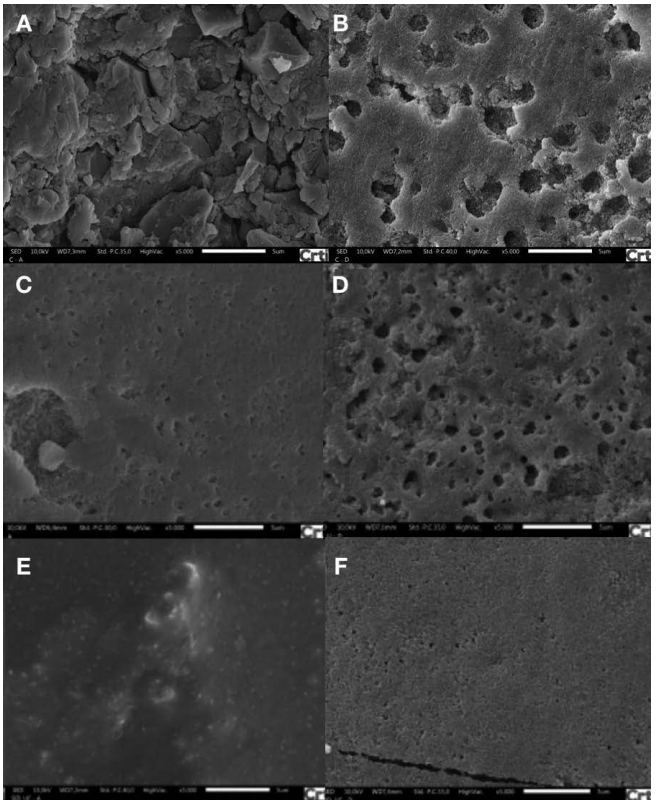

Figure 3. *Photomicrographs of dentin surfaces (5000×): (A) DDS "fresh" dentin; (B) dentin DDS after prophylaxis and application of the adhesive; (C) IDS.U after applying the adhesive; (D) IDS.U after sealed surface treatment; (E) IDS.UF after application of the adhesive and flow; and (F) IDS.UF after sealed surface treatment.*

greater than after 24 hours, with a statistically significant difference only for the IDS.UF group. Darr and Jacobsen[25] stated that the resin cement is not properly polymerized in the initial stages of cementation and that disruption of a restoration's cementation can occur during immediate finishing procedures. According to the authors, restorations may be vulnerable in the first 24 hours. Moreover, factors such as the curing mechanism, choice of adhesive system, and polymerization device can affect the degree of conversion of the cements and, consequently, influence the mechanical properties and clinical performance.[26] Hence, the probable explanations for the increase in the bond strength values over time in the present work are the late curing of the cement, with better polymerization after 3 months, and consequent maturation of the adhesive area. And especially for the IDS.UF group, due to the flow resin providing a more homogeneous sealing of dentin (occluded dentinal tubules), as could be observed in the SEM, the referred maturation of the adhesive layer probably occurred under more favorable conditions, such as by the possible absence of moisture. A strong point also of the present study is that the treatments of the sealed

dentin surface, used before the application of resin cement, involved materials and equipment commonly available in dental offices, which improves the clinical applicability of the research protocols. Furthermore, pumice prophylaxis and applying phosphoric acid to the sealed surface for cleaning purposes are common procedures before cementing indirect restorations. A recent study[4] compared the treatment of sealed surfaces with different IDS strategies before applying resin cement for shear tests. They found that there were no statistical differences in bond strength by comparing pumice-only or pumice plus silica coating and silane. Corroborating the idea that simpler and more common procedures may be sufficient for the treatment of sealed dentin before adhesive cementation.

The analysis by SEM revealed that the IDS.U group presented exposure of dentinal tubules after the treatment of the sealed surface, while the IDS. UF group, certainly due to the flow resin layer, kept the dentin protected with the tubules properly sealed. The exposure at IDS.U can be explained by the low film thickness of the unfilled universal adhesive, which creates a surface that is more vulnerable to mechanical stress resulting from the pumice application.[33] This fact confirms the tested hypothesis that the flow resin layer improves the quality of the dentin sealing, as it acts as additional protection to the substrate and the adhesive area. According to Hironaka and others,[19] the resin coating technique really protects the adhesive layer of the IDS, which is subject to degradation, and also enables a better diffusion of the resin cement. Therefore, applying a layer of flow resin can indeed be considered advantageous in the universal adhesive IDS technique evaluated here.

In the present study, we opted for the use of bovine teeth due to the advantages such as: ease of obtaining the teeth in adequate condition, that is, without carious lesions and enamel defects; greater uniformity found in bovine teeth when compared to human teeth; better standardization of samples; and greater area of flat surface, which makes it possible to make a larger number of samples per tooth and improve the performance of the micro-shear bond strength test.[27] Furthermore, comparative studies between human and bovine teeth, which evaluated the bond strength in enamel and dentin, did not indicate significant differences between them.[28] In this way, bovine teeth are seen as a reliable alternative for carrying out the tests proposed in the present study.

Another important factor regarding the tests performed here consists of the characteristics of the smear layer that can also influence the bond strength, which is why it must be standardized in all specimens. In accordance with what is described in the literature, all samples received a final preparation with 600-grit sandpaper, which simulated a dentin preparation performed by fine-grained diamond-tip wear instruments. The standard of laboratory research was followed, with the samples stored in distilled water at 37°C for 24 hours.[29]

The micro-shear tests, initially addressed in the work of Shimada and others,[30] are designed for the evaluation of the bond strength of materials to the dental structure, being used mainly for friable materials, as is the case of cements, which would be damaged if they were submitted to the protocols required to perform the microtensile test. In a comparative analysis, micro-shear is more reliable and advantageous than the traditional shear test, since it evaluates adhesive areas smaller than 3 mm^2. This makes it possible to place several specimens on a single tooth, in addition to requiring a smaller amount of material, which reduces the likelihood of bubbles and irregularities, which could compromise the test. Another important factor is the concentration of stresses in the adhesive area, which promotes a considerable reduction in the percentage of cohesive failures in material and substrate. In fact, in the present study, there was a predominance of adhesive-type fracture patterns in all groups, which was expected, therefore, by the type of test used.

As the main limitations of the present study, one must consider the great variability of the data, and also the difficulty of external validity of the results inherent to *in vitro* studies. Besides that, the micro-shear bond strength test requires a critical execution technique, due to the fact that the positioning of double-sided guides, starch tubes, and samples in the universal testing machine can compromise data collection. Nevertheless, the findings of this laboratory research are important precursors for the development of other studies, especially controlled clinical trials, in order to obtain better scientific evidence for the definition of a safe and reliable protocol for the cementation of indirect restorations on dentin surfaces immediately sealed with universal adhesive and flow resin.

CONCLUSIONS

According to the present work, it can be concluded that the immediate dentin sealing techniques evaluated in the present study did not promote values of bond strength different from the control group (DDS), either in the 24-hour period or in the 3-month period. In the group that used flow resin after sealing with Single Bond Universal (IDS.UF) there was a significant increase in the bond strength values after 3 months, when compared to the time of 24 hours. The qualitative analysis of the fracture pattern showed a predominance

of adhesive-type fractures. In the IDS.UF group, the flow resin promoted a more regular surface and the dentinal tubules were effectively sealed.

Acknowledgments

We would like to thank the team of the Biomechanics Laboratory of the Dental School of the Federal University of Goiás and the CRTI - Regional Center for Technological Development and Innovation for their support in carrying out this study.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of the institution in which it was carried out.

Conflict of Interest

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

(Accepted 4 September 2021)

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Influence of Irradiance and Exposure Times on the Mechanical and Adhesive Properties of Universal Adhesives with Dentin

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Clinical Relevance

Better bond strength to dentin and degree of conversion were observed when an adhesive was light-cured using a 5 s * 3200 mW/cm^2, which is in agreement with clinician preference for simplification.

SUMMARY

Objectives: This study evaluated the influence of irradiance/exposure time on the Knoop hardness (KHN) and polymer cross-linking density (PCLD), as well as microtensile bond strength (μTBS), nanoleakage (NL), and *in situ* degree of conversion (DC) of universal adhesives.

Methods and Materials: Two universal adhesive

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http://doi.org/10.2341/21-042-L

systems, Clearfil Universal Bond Quick (CUQ) and Scotchbond Universal Adhesive (SBU), were light-cured using various irradiance/exposure times: 1400 mW/cm² for 5 s (1400*5); 1400 mW/cm² for 10 s (1400*10); 3200 mW/cm² for 5 s (3200*5); and 3200 mW/cm² for 10 s (3200*10). Adhesive disks from each group were used to measure PCLD by KHN. One hundred and twenty-eight human molars were randomly assigned to 16 groups according to the following variables: adhesive system vs adhesive strategies vs radiance/exposure times. After restoration, specimens were sectioned into resin-dentin sticks and tested for μTBS, NL, and DC. The data from PCLD (%), KHN, μTBS (MPa), NL (%), and DC (%) data were subjected to ANOVA and Tukey's test (α=0.05).

Results: Significant reductions in KHN, μTBS, and DC (p=0.00001) values and an increase in NL and PCLD (p=0.00001) values were observed for 3200*10 when compared with other groups. Higher KHN, μTBS, and DC (p=0.000001) values were observed for 3200*5 in comparison with the other groups. The 1400*5 (7 J/cm²) and 1400*10 (14 J/cm²) groups showed intermediate values (p=0.000001).

Conclusion: Although similar results in terms of hardness, polymer cross-linking density and nanoleakage were observed when 5 seconds at 3200 mW/cm² and 10 seconds at 1400 mW/cm² groups were compared, the use of higher irradiance (3200 mW/cm²) for only 5 seconds showed better results in terms of bond strength and degree of conversion for both universal adhesives to dentin. The prolonged exposure time (10 seconds) at the higher irradiance (3200 mW/cm²) showed the worst results.

INTRODUCTION

Current bonding strategies are classified according to how adhesive systems interact with the smear layer, and they are divided into the etch-and-rinse (ER) strategy and self-etch (SE) strategy.[1] However, there is a trend among manufacturers to simplify bonding procedures to satisfy the demand for adhesive procedures that are faster, less technique-sensitive, and more user-friendly.[1]

In this sense, several manufacturers launched in the market "Universal" adhesive systems, which provide dentists with the choice of selecting the adhesion strategy—ER, SE, or an alternative "selective enamel etching," which is a combination of ER strategy on enamel and SE strategy on dentin.[2-4] Universal adhesives are single one-bottle, no-mix adhesive systems that perform equally well with any adhesion strategy and bond adequately to the tooth structure as well as to different restorative materials.[3,5]

However, all simplified one-bottle adhesives are very complex blends of hydrophilic and hydrophobic monomers, water, solvents, and photoinitiators.[6] Consequently, the polymerization of simplified one-bottle adhesives may be adversely affected by the remaining solvent[7,8] and water,[9] mainly because the complete evaporation of solvents after application is clinically difficult.[10] Therefore, simplified one-bottle adhesives are associated with a lower polymerization pattern as compared with multi-step adhesives, which usually include a solvent-free adhesive as the final step.[11]

Although little attention has been given to the polymerization of adhesive systems,[12] some studies have shown that it is possible to increase the adhesive performance as well as reduce the permeability by applying a prolonged exposure time during adhesive light-curing of resin-dentin bond sticks.[11,13-17] However, this increases chair time, which does not comply with the clinician's preference for simplification. Recently, high-irradiance, third-generation, polywave LED curing units were introduced, capable of reaching more than 3000 mW/cm². These new light-curing devices were launched as a solution to increase the restoration thickness and potentially allow shorter polymerization times to achieve optimal photocuring of restorative materials.[18]

The manufacturers of these polywave devices based the effective polymerization process on the exposure reciprocity law,[19,20] and suggest using a curing light that emits a high irradiance could reduce the exposure time. Thus, it is possible that these polywave devices achieve sufficient polymerization using a shorter curing time, because of the increased irradiance.[21] However, most dental adhesive manufacturers indicate a single exposure duration regardless of the material type, shade, or clinical distance from the tip, recommending the specific exposure times for their products that often do not match those of the light manufacturers.[12]

To the best of our knowledge, no study has addressed the effect of different radiant exposures on the mechanical and bonding properties of universal adhesives using a high-power polywave device. Thus, this study aimed to evaluate the influence of radiant exposure on the Knoop hardness (KHN) and polymer cross-linking density (PCLD), as well as the dentin bonding properties (microtensile bond strength [μTBS], nanoleakage [NL], and *in situ* degree of conversion [DC]) of universal adhesives. The null hypotheses tested were that irradiance and exposure

time do not affect (1) the Knoop hardness and polymer cross-linking density, as well as (2) μTBS and DC and (3) NL of universal adhesives bonded to dentin.

METHODS AND MATERIALS

Experimental Design and Calibration Procedures

Two commercial universal adhesive systems were used: Clearfil Universal Bond Quick (CUQ, Kuraray Noritake, Tokyo, Japan) and Scotchbond Universal Adhesive (SBU, 3M Oral Care, St Paul, MN, USA). The detailed composition and batch number of the adhesives are described in Table 1. An LED light-curing unit (Valo, Ultradent Products, Salt Lake City, UT, USA) was used at an irradiance of 1400 mW/cm^2 or 3200 mW/cm^2 for 5 or 10 seconds, resulting in different delivered energy levels (7-32 J/cm^2).

These values were determined using a laboratory-grade spectroradiometer (USB 2000, Ocean Optics, Dunedin, FL, USA) previously calibrated using a NIST-traceable light source and connected to a 6" integrating sphere (Labsphere, North Sutton, NH, USA). For this purpose, the light-emitting area tip end was positioned 1 mm away at the entrance of the integrating sphere, so that all light emitted from the unit was captured. The spectral power measurements were obtained using software (SpectraSuite, v2.0.146, Ocean Optics), where the integrated area was between 350 and 550 nm, which also provided the total emitted power value for that wavelength range. Radiant emittance values of each exposure mode (mW/cm^2) were determined as the total measured power value was divided by the light-emitting area of the distal tip end. This value was then multiplied by the light exposure duration to derive the value of radiant exposure applied to each tooth surface for each light output mode (J/cm^2).

Sample Size

The main outcome of the present study was dentin bond strength. The mean bond strength values of universal adhesives applied to the dentin were considered in the sample size calculation.[4] According to previous literature, the mean bond strength (± standard deviation) of an evaluated universal adhesive was 37 ± 4.0 MPa. To detect a difference of 6 MPa among the tested groups, using $\alpha = 0.05$, a power of 80%, and a two-sided test, the estimated minimum sample size was 8 teeth in each group. The same number of teeth was used for all bonding properties evaluated.

Table 1: *Adhesive Systems, Batch Number, Composition, Groups, and Application Mode*

Adhesive Systems/Batch Number	Composition	Groups	Application Mode	
			Etch-and-rinse	Self-etch
Clearfil Universal Bond Quick (CUQ) Kuraray/ CD0012	10-MDP, BisGMA, HEMA, hydrophilic amide resin monomers, colloidal silica, silane coupling agent, NaF, camphorquinone, ethanol, water	1400 mW/cm^2 for 5 s (1400*5 = 7 J/cm^2) 1400 mW/cm^2 for 10 s (1400*10 = 14 J/cm^2)	1. Apply etchant for 15 s. 2. Rinse thoroughly. 3. Blot excess water; 4. Apply adhesive as for the self-etch mode	1. Apply adhesive to the entire surface with a microbrush and rubbing. No waiting time is required. 2. Dry by blowing mild air for 5 s until the adhesive did not move. 3. Light cure according to experimental groups
Scotchbond Universal (SBU) 3M Oral Care/638367	10-MDP, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, nanofiller, ethanol, water, initiators, silane	3200 mW/cm^2 for 5 s (3200*5 = 16 J/cm^2) 3200 mW/cm^2 for 10 s (3200*10 = 32 J/cm^2)	1. Apply etchant for 15 s. 2. Rinse thoroughly. 3. Blot excess water. 4. Apply adhesive as for the self-etch mode.	1. Applied the adhesive to the entire preparation and left undisturbed for 20 s. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent evaporates completely. 3. Light cure according to experimental groups.

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; bis-GMA, bisphenol glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate.

Knoop Hardness Test for Polymer Cross-Linking Density

The adhesive disks for the KHN and PCLD tests were produced based on the combination of the main variables, that is: (1) *Adhesives*: CUQ and SBU; (2) *Irradiance/exposure times*: 1400 mW/cm^2 for 5 seconds (7 J/cm^2), 1400 mW/cm^2 for 10 seconds (14 J/cm^2), 3200 mW/cm^2 for 5 seconds (16 J/cm^2), and 3200 mW/cm^2 for 10 seconds (32 J/cm^2); and (3) *Immersion in ethanol*: measurement was performed *before* and *after* immersion in ethanol.

Five adhesive disks of each material were produced in a brass mold 5.0 mm in diameter and 1.0 mm in height (Odeme Prod Odont, Joaçaba, SC, Brazil).[22] The adhesive was dripped into the mold, until filling it completely. The air bubbles trapped in the adhesives were removed with a microbrush. Then, the solvent was evaporated using an air-water syringe for 40 seconds. A polyester Mylar strip was placed on top of the adhesive, which was light-cured according to each irradiance, time, and adhesive group. For standardizing the light-cured procedures, the Valo unit was fixed in a clamp with the emitting end of the light guide 1 mm away from the top surface. After light-curing, the specimens were stored in a dry environment at 37°C for 24 hours.

For measurement of Knoop hardness, indentations were made in the light-cured top surface with a Knoop indenter (KHN, Shimadzu, Kyoto, Japan) using a 10 g load for 15 seconds. Three indentations were made in each specimen, and these values were averaged for statistical purposes. After the first KHN measurement, specimens were stored in a 100% ethanol solution at 37°C for 24 hours, and then the second KHN measurement was performed. The polymer cross-linking density was estimated by the softening effect of the ethanol, that is, by the decrease in hardness.[22]

Selection and Preparation of Teeth

One hundred and twenty-eight extracted, caries-free human molars were used. The teeth were collected after obtaining the patients' informed consent. The teeth were disinfected in 0.5% chloramine, stored in distilled water, and used within 6 months after extraction. The teeth were sectioned parallel to the occlusal surface using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water-cooling to expose the mid-coronal dentin. A smear layer was standardized for all specimens by grinding each flat dentin surface with #600-grit silicon carbide paper (SiC) under running water for 60 seconds.

Experimental Design

The teeth were then randomly assigned to 16 groups (n=8 dentin specimens for μTBS, NL, and DC) based on the combination of the main variables, that is: (1) *Adhesive*: CUQ and SBU; (2) *Adhesive strategies*: two-step etch-and-rinse (ER) and one-step self-etch (SE) approaches; and (3) *Irradiance/exposure times*: 1400 mW/cm^2 for 5 seconds (7 J/cm^2), 1400 mW/cm^2 for 10 seconds (14 J/cm^2), 3200 mW/cm^2 for 5 seconds (16 J/cm^2), and 3200 mW/cm^2 for 10 seconds (32 J/cm^2). The composition, application mode, and batch numbers are listed in Table 1.

Restorative Procedures

For the ER strategy, a 37% phosphoric acid gel (Condac, FGM Dental Products, Joinville, SC, Brazil) was applied to the dentin surfaces for 15 seconds, followed by rinsing with water for 30 seconds and air-drying for 5 seconds. For the SE strategy, the dentin was not conditioned. For both strategies, the adhesives were applied according to the manufacturer's instructions, and light-cured according to each irradiance/exposure time. For standardizing the light-cured procedures, the Valo unit was fixed in a clamp with the emitting end of the light guide 1 mm away from the occlusal surface. To achieve the exposure times when the device was in high irradiance mode and considering the Valo was set to 3 seconds, the curing time was set to 5 (3 + 2) and 10 (3 × 3 +1) seconds, with the light spot performed immediately in sequence.

The resin composite buildups (DA4, Opallis, FGM, Joinville, Brazil) were then constructed incrementally (three 1.5-mm increments) and each increment was light-cured for 40 seconds each using the same LED light-curing unit (Valo, Ultradent Products) at 1000 mW/cm^2 (40 J/cm^2). A single operator performed all the bonding procedures.

After storage in distilled water at 37°C for 24 hours, the specimens were sectioned longitudinally in the mesio-distal and buccal-lingual directions across the bonded interface, using the low-speed diamond saw to obtain resin-dentin bonded sticks with a cross-sectional area of approximately 0.8 mm^2, as measured with digital calipers (Digimatic Caliper, Mitutoyo, Tokyo, Japan). The number of resin-dentin bonded sticks showing pretest failure (PTF) during specimen preparation was recorded for each tooth.

The resin-dentin bonded sticks obtained for each tooth were randomly distributed as follows: three and two resin-dentin bonded sticks from each tooth and each experimental condition were evaluated, respectively, for NL and DC within adhesive/hybrid layers; the rest of the specimens were tested for μTBS testing. As the

mean μTBS, NL and DC of all resin-dentin bonded sticks from the same tooth were averaged for statistical analyses, and the sample size was eight teeth per group for each experimental group and for each test evaluated (μTBS, NL, and DC tests).

Microtensile Bond Strength Testing

Resin-dentin bonded sticks were attached to a Geraldeli's jig[23] with cyanoacrylate adhesive and tested under tension (Kratos Dinamometros; Cotia, SP, Brazil) at 0.5 mm/min until failure. The μTBS values (MPa) were calculated by dividing the load at failure by the cross-sectional bonding area. The failure modes of the resin-dentin bonded sticks were classified as either cohesive ([C], failure exclusively within the dentin or the resin composite), or adhesive/mixed ([A/M], failure at the resin-dentin interface, or failure at the resin-dentin interface with partial cohesive failure of the neighboring substrates). This classification was performed under a stereomicroscope (Olympus SZ40; Tokyo, Japan) at 100× magnification. The number of specimens with PTF was low, and because of this, it was not included in the average.

Nanoleakage Evaluation

The resin-dentin bonded sticks were immersed in ammoniacal silver nitrate solution according to the protocol previously described by Tay and others.[24] The resin-dentin bonded sticks were placed in the solution in the dark for 24 hours, rinsed thoroughly in distilled water, and immersed in photodeveloping solution for 8 hours under fluorescent light to reduce silver ions to metallic silver grains within the spaces along the bonded interface. The specimens were polished with wet 600-, 1000-, 1200-, 1500-, 2000-, and 2500-grit SiC paper using a polishing cloth. They were then ultrasonically cleaned, air dried, mounted on stubs, and coated with carbon-gold (MED 010, Balzers Union, Balzers, Liechtenstein). Resin-dentin interfaces were analyzed using a field-emission scanning electron microscope operated in backscattered mode (VEGA 3 TESCAN, Shimadzu, Tokyo, Japan). Photomicrographs of representative surface area were taken at 1000× magnification. Three images of each resin-dentin bonded stick were captured.[25,26] The relative percentage of NL within the adhesive and hybrid layers in each specimen was measured in all images using Image J software (National Institutes of Health, Bethesda, MD, USA).[27]

In Situ Degree of Conversion (*in situ* DC) Within Adhesive/Hybrid Layers

The resin-dentin bonded sticks were wet polished, ultrasonically cleaned and positioned in a micro-Raman microscope (XploRA ONE, HORIBA Scientific, Piscataway, NJ, USA), which was first calibrated to zero and then to coefficient values using a silicon sample. The samples were analyzed using a 532-nm diode laser through a 100× air objective. The Raman signal was acquired with 600 lines/mm on a graft centered between 400 and 2000 cm^{-1}. The employed parameters were 100 mW, spatial resolution 3 μm, spectral resolution 1 cm^{-1}, and accumulation time 30 seconds with 5 co-additions. Spectra were obtained at the adhesive-dentin interface at three random sites per bonded stick, within the hybrid layer in the intertubular-infiltrated dentin. Post-processing of the spectra was performed using Opus Spectroscopy Software version 6.5. The average of the values was used for statistical analysis, and the spectra of the uncured adhesives were considered as references.

The ratio of the double-bond content of monomer to polymer in the adhesive was quantified by calculating the ratio derived from the aliphatic C=C (vinyl) absorption (1638 cm^{-1}) to the aromatic C=C absorption (1608 cm^{-1}) signals for both polymerized and unpolymerized samples (n=5). The DC was calculated according to the following formula:

$$\textit{In situ}\ \text{DC}\ (\%) = (1-[R_{\text{cured}}/R_{\text{uncured}}]) \times 100,$$

where "*R*" is the ratio of aliphatic and aromatic peak intensities at 1639 cm^{-1} and 1609 cm^{-1} in cured and uncured adhesives, respectively.[26] In addition, the more intense peaks observed and the corresponding chemical bonds were recorded for all materials.

Statistical Analysis

The mean values of the KHN test were subjected to a three-way ANOVA (adhesive vs irradiance/exposure times vs immersion in ethanol). In addition, the PCLD (%) data were evaluated by two-way ANOVA (adhesive vs irradiance/exposure times).

The mean μTBS (MPa), NL (%), and DC (%) of all bonded sticks from the same tooth were averaged for statistical analyses, ensuring that the experimental unit in the study was the tooth. The number of specimens with PTF was low (ranging between 1.5 to 3%), and because of this, it was not included in the average. The μTBS, NL, and DC mean for each group were obtained from the average of the eight teeth used per group. For the bonding properties, data were subjected to three-way ANOVA (adhesive strategy vs adhesive vs irradiance/exposure times). Finally, for all tests, Tukey's test with a level of significance of 5% was applied.

Table 2: *Knoop Hardness Values (KHN ± Standard Deviation) as an Estimation of Polymer Cross-Linking Density (Δ %) After Immersion in Absolute Ethanol for All Experimental Groups*[a]

Groups	Clearfil Universal Bond Quick						Scotchbond Universal					
	5 s			10 s			5 s			10 s		
	Before	After	Δ (%)	Before	After	Δ (%)	Before	After	Δ (%)	Before	After	Δ (%)
1400 mW/cm²	10.2 ± 0.5 b	5.4 ± 1.5 c	46 B	13.6 ± 1.2 a	8.8 ± 1.4 b	35.4 A	10.1 ± 1.7 b	6.2 ± 1.1 c	44 B	14.2 ± 1.4 a	9.3 ± 0.8 b	35 A
3200 mW/cm²	13.3 ± 1.1 a	8.9 ± 1.2 b	33 A	8.2 ± 1.3 b	2.0 ± 0.6 d	75.1 C	13.7 ± 0.8 a	8.9 ± 1.4 b	35 A	6.4 ± 1.7 c	1.7 ± 0.4 d	73 C

[a] *Different lowercase letters indicate statistically different means in KHN (three-way repeated measured ANOVA and Tukey's test; p<0.005); different uppercase letters indicate statistically different means in estimation of polymer cross-linking density (two-way ANOVA and Tukey's test; p<0.005).*

RESULTS

Knoop Hardness Test for Polymer Cross-Linking Density

The KHN and the estimation of the PCLD are shown in Table 2. Regarding the Knoop values, the cross-product interaction was not significant, as was the main factor adhesive (Table 3). However, the primary factors of irradiance/exposure times and immersion in ethanol were considered statistically significant (Table 3; p=0.000001 and p=0.000001, respectively). Higher KHN values were observed for the 3200*5 (16 J/cm²) and 1400*10 (14 J/cm²) groups, while lower KHN values were observed for 3200*10 (32 J/cm²) when compared to the other groups (Table 2). The 1400*5 (7 J/cm²) group showed intermediate KHN values (Table 2). In contrast, for all groups, a significant decrease in KHN was observed after ethanol immersion (Table 2).

However, a significant decrease in PCLD was observed after ethanol immersion in all groups (Table 2; p=0.00001), with a higher percentage of reduction observed in the 3200*10 (32 J/cm²) group. In contrast, a lower percentage of reduction in the PCLD was measured for the 3200*5 (16 J/cm²) and 1400*10 (14 J/cm²) groups. The 1400*5 (7 J/cm²) group showed intermediate PCLD reduction values (Table 2; p=0.000001).

Microtensile Bond Strength (μTBS)

The majority of the fracture pattern was adhesive and mixed failures (data not shown). Regarding the μTBS values, the cross-product interaction was not significant, as well as the main factor adhesive strategy (Table 4). However, the primary factors, that is, adhesive and irradiance/exposure time were considered statistically significant (Table 4; p=0.000001 and p=0.000001, respectively).

Higher μTBS values were observed for 3200*5, while lower μTBS values were observed for 3200*10 (32 J/cm²) when compared to the other groups (Table 5). Regarding the other groups, for CUQ, there was no significant difference between 1400*5 (7 J/cm²) and 1400*10 (14 J/cm²). In contrast, for SBU, 1400*5 (7 J/cm²) showed lower μTBS values when compared to 1400*10 (14 J/cm²) (Table 5). Overall, SBU showed higher μTBS values when compared to CUQ (Table 5).

Table 3: *Three-Way ANOVA Results (Knoop Hardness Test Values)*

Source of Variation (*)	SS	DF	MS	F	P
Intercept	6322.035	1	6322.035	2267.423	0.000000
Adhesive	0.425	1	0.425	0.152	0.697569
Strategy	502.453	1	502.453	180.207	0.000001
Irrad/time	608.338	3	202.779	72.728	0.000001
Adhesive*Strategy	0.275	1	0.275	0.099	0.754523
Adhesive*Irrad/Time	9.840	3	3.280	1.176	0.325738
Strategy*Irrad/Time	2.340	3	0.780	0.280	0.839815
Adhesive*Strategy*Irrad/Time	2.729	3	0.910	0.326	0.806373
Error	178.445	64	2.788		

Abbreviations: SS, sum of squares; DF, degrees of freedom; MS, mean squares; F, f obtained; p, probability.

Table 4: *Three-Way ANOVA Results (Microtensile Bond Strength Test Values)*

Source of Variation (*)	SS	DF	MS	F	P
Intercept	193565.2	1	193565.2	11190.70	0.000000
Adhesive	1350.5	1	1350.5	78.08	0.000001
Strategy	199.3	1	199.3	11.53	0.095060
Irrad/time	9200.9	3	3067.0	177.31	0.000001
Adhesive*Strategy	0.1	1	0.1	0.01	0.938365
Adhesive*Irrad/Time	115.5	3	38.5	2.23	0.088998
Strategy*Irrad/Time	82.0	3	27.3	1.58	0.198122
Adhesive*Strategy*Irrad/Time	15.1	3	5.0	0.29	0.831774
Error	1937.3	112	17.3		

Abbreviations: SS, sum of squares; DF, degrees of freedom; MS, mean squares; F, f obtained; p, probability.

Nanoleakage Analysis (NL)

The cross-product interaction as well as the main factors of adhesive strategy and adhesive were not significant (Table 6). However, the primary factor of irradiance/exposure time was considered statistically significant (Table 6; p=0.000007). A significantly higher NL value was observed for the 3200*10 group than in the other groups (Table 7 and Figure 1). In addition, a closer view regarding the NL of the ER strategy values showed that significant and lower NL values were observed when 1400*10 (14 J/cm^2) was compared to 1400*5 (7 J/cm^2) (Table 7 and Figure 1). Figure 1 showed the representative photomicrographs obtained for all experimental groups.

Table 5: *Means of Microtensile Bond Strength Values (MPa ± Standard Deviation) for All Experimental Groups*[a]

Groups	Clearfil Universal Bond Quick				Scotchbond Universal			
	Etch-&-rinse		Self-etch		Etch-&-rinse		Self-etch	
	5 s	10 s	5 s	10 s	5 s	10 s	5 s	10 s
1400 mW/cm^2	33.62 ± 4.6 cd	36.87 ± 3.9 c	31.82 ± 4.8 cd	35.15 ± 3.9 c	36.44 ± 3.7 c	46.58 ± 4.5 b	35.39 ± 4.1 c	45.23 ± 4.0 b
3200 mW/cm^2	47.27 ± 4.5 b	25.83 ± 3.9 e	47.03 ± 3.9 b	23.24 ± 4.4 e	54.79 ± 4.5 a	31.80 ± 4.5 cd	53.38 ± 3.3 a	27.39 ± 2.6 de

[a]*Different letters indicate statistically different means (three-way ANOVA and Tukey's test;* p<*0.005).*

Table 6: *Three-Way ANOVA Results (Nanoleakage Test Values)*

Source of Variation (*)	SS	DF	MS	F	P
Intercept	459060.0	1	459060.0	27619.80	0.000000
Adhesive	0.0	1	0.0	0.00	0.988980
Immersion in ethanol	23.7	1	23.7	1.43	0.234974
Irrad/time	496.4	3	165.5	9.96	0.000007
Adhesive*immersion in ethanol	0.5	1	0.5	0.03	0.865061
Adhesive*Irrad/Time	56.1	3	18.7	1.13	0.342109
Immersion in ethanol*Irrad/Time	36.4	3	12.1	0.73	0.536657
Adhesive*immersion in ethanol*Irrad/Time	83.9	3	28.0	1.68	0.174837
Error	1861.5	112	16.6		

Abbreviations: SS, sum of squares; DF, degrees of freedom; MS, mean squares; F, f obtained; p, probability.

Table 7: *Means of Silver Nitrate Deposition (% ± Standard Deviation) for All Experimental Groups*[a]

Groups	Clearfil Universal Bond Quick				Scotchbond Universal			
	Etch-&-Rinse		Self-etch		Etch-&-Rinse		Self-etch	
	5 s	10 s	5 s	10 s	5 s	10 s	5 s	10 s
1400 mW/cm²	11.20 ± 1.9 b	8.23 ± 1.6 a	10.43 ± 1.9 ab	7.35 ± 1.9 a	11.09 ± 1.8 b	6.31 ± 1.7 a	9.37 ± 1.7 ab	5.26 ± 1.1 a
3200 mW/cm²	9.54 ± 1.8 ab	16.38 ± 1.6 c	7.27 ± 1.6 a	15.73 ± 1.5 c	8.39 ± 1.6 ab	15.33 ± 1.7 c	6.11 ± 1.8 a	13.29 ± 1.9 c

[a]*Different letters indicate statistically different means (three-way ANOVA and Tukey's test;* p<*0.005)*

In Situ Degree of Conversion (*in situ* DC) Within Adhesive/Hybrid Layers

Regarding the DC values, the cross-product interaction was not significant, in addition to the main factor adhesive strategy (Table 8). However, the primary factors, that is, adhesive and irradiance/exposure time were considered statistically significant (Table 8; p=0.00002 and p=0.00001, respectively).

Higher DC values were observed for 3200*5, while lower DC values were observed for 3200*10 (32 J/cm²) when compared to the other groups (Table 9). When 1400*5 (7 J/cm²) and 1400*10 (14 J/cm²) were compared, no significant difference was observed for DC. However, overall, SBU showed a majority of higher DC values when compared to those of CUQ (Table 9).

DISCUSSION

Adhesive systems are considered resin-based materials and require light-curing to achieve maximum mechanical properties.[12] However, only a few studies have been carried out to evaluate the effect of polymerization on improving the adhesive properties of dentin.[11,13-17] It seems that, due to the non-opaque yellowish color aspects of the adhesive systems arising from the low amount of filler added,[28] dentists and manufacturers tend to neglect the polymerization time or the irradiance necessary to achieve the correct adhesive polymerization when applied to the cavity.[12] Some studies have reported that the exposure time recommended for dental adhesives is not adequate to obtain an optimal polymerization, even under *in vitro* conditions.[11,13-17]

The polymerization reaction of adhesive systems requires a certain amount of quantum energy to activate the photoinitiator so that it can react with a co-initiator to produce free radicals.[29,30] Therefore, the increase in the amount of quantum energy, through higher irradiance or by increasing the exposure times,

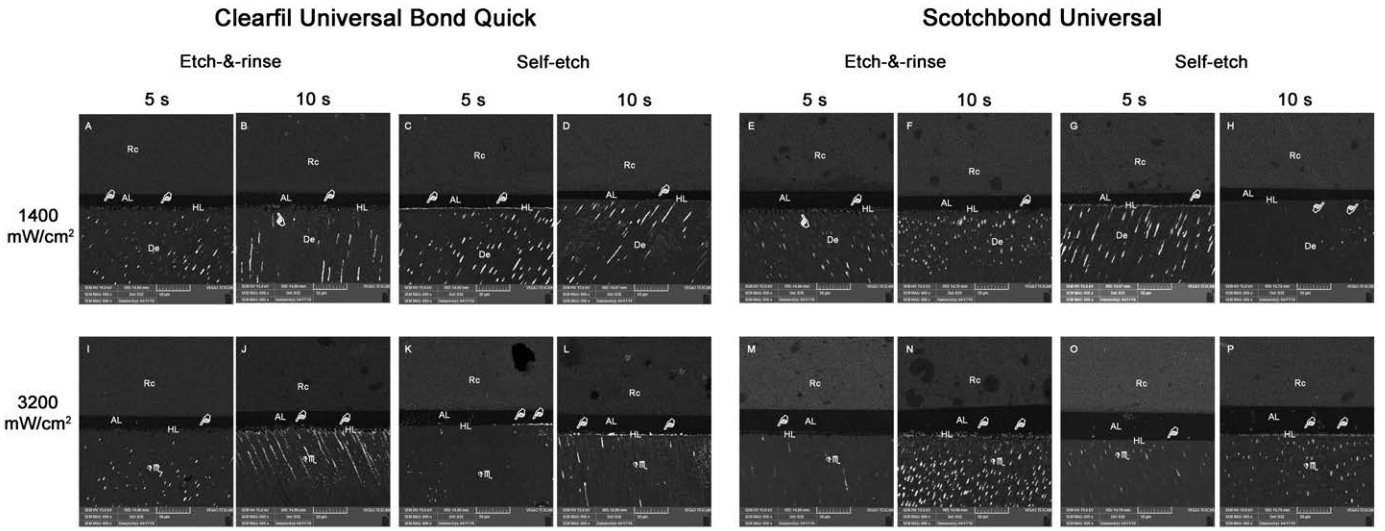

Figure 1. *Photomicrographs obtained by backscattered SEM mode of all experimental groups. Independent of the adhesive system used, it is possible to observe a mild silver deposit at the resin-dentin interface for 1400*10 and 3200*5 groups (hand indicator). For both adhesive strategies and adhesive systems used, the 3200*10 groups demonstrated a more pronounced silver nitrate infiltration (hand indicator). Rc, resin composite; AL, adhesive layer; HL, hybrid layer; De, dentin (micron bar = 50 mm; original magnification = 1000×).*

Table 8: *Three-Way ANOVA Results (*In Situ *Degree of Conversion Within Adhesive/Hybrid Layers Test Values)*

Source of Variation (*)	SS	DF	MS	F	P
Intercept	14746.03	1	14746.03	5347.859	0.000000
Adhesive	69.65	1	69.65	25.259	0.000002
Strategy	0.00	1	0.00	0.000	1.000000
Irrad/time	1333.43	3	444.48	161.196	0.000001
Adhesive*Strategy	0.00	1	0.00	0.000	1.000000
Adhesive*Irrad/Time	0.00	3	0.00	0.000	1.000000
Strategy*Irrad/Time	3.85	3	1.28	0.465	0.707327
Adhesive*Strategy*Irrad/Time	0.00	3	0.00	0.000	1.000000
Error	308.83	112	2.76		

Abbreviations: SS, sum of squares; DF, degrees of freedom; MS, mean squares; F, f obtained; p, probability.

leads to the formation of more free radicals, which initiates polymerization.[29] This may provide room for the formation of a high-molecular-weight cross-linked polymer.[8,13] This better polymerization leads to a less permeable[8,11,14] and more resistant hybrid layer to water degradation.[13,16]

It was observed that an increase in irradiance (3200*5) or exposure time (1400*10) that results in very similar delivered energy (16 and 14 J/cm^2), when compared to the 1400*5 group, significantly improved the DC inside the hybrid layer along with the immediate microhardness (as an indirect measurement of the DC), in agreement with previous studies published with an older generation of adhesive systems.[11,13-17] This could be attributed to a higher amount of quantum energy delivered when an increase of irradiance (3200*5) or exposure time (1400*10) was applied. The greater the amount of quantum energy, the better the polymer formed.[12,30]

Despite all groups experiencing a considerably decreased percentage of PCLD after immersion in ethanol, a significantly higher percentage of PCLD was observed for the 1400*5 (7 J/cm^2) group when compared to the 3200*5 (16 J/cm^2) or 1400*10 (14 J/cm^2) groups. The PCLD is considered an indirect test to measure a polymer network by softening.[22,31,32] This is accepted as an appropriate test, because highly cross-linked polymers are more resistant to degradation and solvent uptake, whereas linear polymers present more space and pathways for solvent molecules to diffuse within their structure.[33] When an inadequate curing time is applied, there is a risk of lower cohesion in the network due to less cross-linking and secondary forces.[12] Therefore, the results obtained in the 1400*5 (7 J/cm^2) group could be attributed to a lower cross-linking density, due to the insufficient energy delivered.

Another factor that cannot be ruled out is the increase in the temperature produced by the high-energy polywave light-curing units,[18,34-36] maximized by an increase in irradiance (3200*5 [16 J/cm^2]) or by prolonged exposure time (1400*10 [14 J/cm^2]). Mouhat and others[34] measured the superficial temperature of different radiant exposures. At 7 J/cm^2, a radiant exposure similar to that obtained for the 1400*5 group, the temperature values measured were around 39.6°C. Otherwise, an increase of 3.2 to 4.1°C was observed when radiant exposure ranged between 14 and 15 J/cm^2. These last radiant exposures are similar to those produced by the 3200*5 (16 J/cm^2) and 1400*10 (14 J/cm^2) groups.

Table 9: *Means of* In Situ *Degree of Conversion Values (% ± Standard Deviation) for All Experimental Groups*[a]

Groups	Clearfil Universal Bond Quick				Scotchbond Universal			
	Etch-&-rinse		Self-etch		Etch-&-rinse		Self-etch	
	5 s	10 s	5 s	10 s	5 s	10 s	5 s	10 s
1400 mW/cm^2	56.49 ± 1.0 cd	60.34 ± 1.9 c	55.44 ± 1.8 cd	59.89 ± 1.2 c	64.21 ± 1.8 b	65.14 ± 1.4 b	58.33 ± 1.7 c	59.22 ± 2.2 c
3200 mW/cm^2	67.25 ± 1.0 b	51.29 ± 1.3 d	65.38 ± 1.4 b	50.31 ± 1.7 d	71,36 ± 1.0 a	45.32 ± 1.3 e	69.60 ± 1.3 a	45.56 ± 1.1 e

[a] *Different letters indicate statistically different means (three-way ANOVA and Tukey's test;* p<*0.005).*

In addition, the increase in temperature can be responsible for favoring the evaporation of solvents from the material,[13] as is known from the benefits of heat in the evaporation of solvents, mainly when using warm-air drying for solvent evaporation.[37,38] Reis and others,[13] based on thermogravimetric analysis, hypothesized that the extra heat and energy produced by the increase in the light exposure could have favored the evaporation of solvent and water, but also increased the degree of conversion of the material,[8,13,14,16] thereby reducing the amount of residual low molecular weight monomers and oligomers.

All of these together might be considered sufficient reasons for the higher resin–dentin bond strengths observed in the present investigation, when an increase in irradiance (3200*5 [16 J/cm^2]) or prolonged exposure time (1400*10 [14 J/cm^2]) was applied in comparison to the 1400*5 (7 J/cm^2) group for both universal adhesives and adhesive strategies, leading the authors to reject all null hypotheses.

Despite a very close radiant exposure for the 3200*5 (16 J/cm^2) and 1400*10 (14 J/cm^2) groups, the former showed a higher DC value inside the hybrid layer and a higher μTBS when compared to the latter. For both adhesives and adhesive strategies, the irradiance was more important for improving the adhesive properties than the increase in polymerization time. In other words, for both adhesive systems used, it seems that the higher irradiance led to an optimum rate of initiation that produced the highest quantum yield and, consequently, better mechanical properties. On the other hand, this may not be enough to form a highly cross-linked polymer, as both groups showed a similar percentage of PCLD. Future studies need to be conducted to confirm the present hypothesis.

However, the most unexpected result was obtained for the 3200*10 group. All properties evaluated were negatively influenced by the higher radiant exposure used (32 J/cm^2). It is known that during the photopolymerization of monomers, a significant increase in temperature occurs in a short exposure time, even in thin films such as adhesive systems, especially when ultrafast polymerization is used.[39] As a result, the molecular mobility increases, particularly in the gelation stage, thus lifting some of the migration restrictions of the reactive species, which are known to be primarily responsible for the premature ending of the polymerization.[39]

Independent of the radiant exposure, among high-power light-curing units, the Valo device produces the greatest increase of temperature[18,34-36] which seemed to directly affect the results of the 3200*10 group. The use of radiant exposure at approximately 32 J/cm^2 significantly increased the superficial temperature to 48.2°C, while an increase of 5°C was seen for 14-16 J/cm^2 and 8.6°C for 7 J/cm^2 when compared with the superficial temperatures evaluated in the other groups of this study.[34] Thus, we speculate that this increase in temperature, due to a large amount of heat received in the 3200*10 group, substantially impaired the adhesive system polymerization, leading to low KHN values and a greater reduction in the percentage of PCLD as well as low μTBS and higher NL values, leading to further rejection of all null hypotheses.

Moreover, the increased irradiance of the 3200*10 group not only produced adhesive interfaces with a high number of potential failures, but the elevated temperature during polymerization may also result in the generation of heat within the tooth, pulp chamber, and surrounding tissues.[18,34-36] For example, a higher temperature has been observed in the pulp chamber when an adhesive was light-cured in comparison with light-curing of composite or base/liner materials.[35,40] Therefore, when an adhesive is applied as the first layer in medium or deep cavities, during the light-curing procedure, the tooth should be air-cooled during the photocuring procedure, or an interval of 1-2 seconds should be included after every 5-10 seconds of light exposure, as indicated by Strassler and Price.[41] It is worth mentioning that the manufacturer of Valo did not recommend 10 seconds for light-curing any resin-based material when applied in a "plasma/turbo" mode.

In this study, two universal adhesive systems were tested, with SBU demonstrated to have higher values in terms of bond strength and DC values when compared to CUQ. The former is the only universal adhesive to be previously evaluated, which contains two functional monomers: 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) and methacrylate-modified polyalkenoic acid copolymer[42,43] which potentiate the chemical interaction of the SBU with the tooth structure.[44] In addition, the application procedures between both materials are different. CUQ is used without waiting for the adhesive to interact with the bonding substrate (the "no-waiting" concept).[45] This can influence the DC results, due to the low time for solvent evaporation, as was observed in the present study, even with increasing radiant exposure (7 to 14-16 mW/cm^2). However, the increase in the DC only affected the bond strength results, since no significant difference was observed in the NL or PCLD results when both adhesives were evaluated.

Finally, it is worth mentioning that, according to a review published by Cadenaro and others,[12] the adequate polymerization of an adhesive has been clearly

correlated with its stability. Therefore, future studies need to be conducted to evaluate the effectiveness of increasing the radiant exposure on the long-term bonding performance of universal bonding adhesives to dentin.

CONCLUSIONS

Similar results in terms of KHN, PCLD and NL were observed when 5 seconds at 3200 mW^2 and 10 seconds at 1400 mW/cm^2 groups were compared. The use of higher irradiance (3200 mW/cm^2) for only 5 seconds showed better results in terms of bond strength and degree of conversion for both universal adhesives to dentin. The prolonged exposure time (10 seconds) at the higher irradiance (3200 mW/cm^2) showed the worst results.

Acknowledgments

This study was performed by Andres F M Cardenas as partial fulfillment of his post-doc stage at the State University of Ponta Grossa (UEPG), Ponta Grossa, PR, Brazil. This study was partially supported by the State Foundation of Support to Research, Scientific and Technological Development of Maranhao (FAPEMA) From State Government of Maranhao Brazil, under grants 002/2019, the National Council for Scientific and Technological Development (CNPq) under grants 303332/2017-4 and 308286/2019-7 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors are grateful for the technical support of the interdisciplinary laboratory CLABMU of State University of Ponta Grossa.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of State University of Ponta Grossa under protocol #3.542.383.

Conflict of Interest

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

(Accepted 10 September 2021)

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Influence of Manganese Oxide on the Esthetic Efficacy and Toxicity Caused by Conventional In-office Tooth Bleaching Therapy

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Clinical Relevance

The improved efficiency and reduced toxicity observed after adding manganese oxide to a bleaching gel with 35% H_2O_2, commonly used for professional therapy, may prevent pulp damage and post-bleaching (in-office) dental sensitivity.

SUMMARY

Objective: This study aimed to evaluate the esthetic efficacy, cytotoxicity, and kinetics of decomposition of hydrogen peroxide (H_2O_2) present in a commercial bleaching gel with 35% H_2O_2 (BG35%) chemically activated with manganese oxide (MnO_2).

Methods and Materials: After incorporating 2 mg/mL, 6 mg/mL, and 10 mg/mL of MnO_2 into BG35%, the stability of pH and temperature of

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http://doi.org/10.234/20-257-L

the products were analyzed. To assess the esthetic efficacy (ΔE and ΔWI), the BG35%s with MnO_2 were applied for 45 minutes on enamel/dentin discs (DiE/D). BG35% or no treatment were used as positive (PC) and negative (NC) controls, respectively. To analyze the cell viability (CV) and oxidative stress (OXS), the same bleaching protocols were performed on DiE/D adapted to artificial pulp chambers. The extracts (culture medium + gel components that diffused through the discs) were applied to pulp cells and submitted to H_2O_2 quantification. BG35% with MnO_2 that showed the best results was evaluated relative to kinetic decomposition of H_2O_2, with consequent generation of free radicals (FR) and hydroxyl radicals ($OH^•$). The data were submitted to the one-way analysis of variance complemented by Tukey post-test (α=0.05). Data on kinetics of H_2O_2 decomposition were submitted to the Student's-*t* test (α=0.05).

Results: All the BG35%s with MnO_2 showed stability of pH and temperature, and the gel with 10 mg/mL of this activator had an esthetic efficacy 31% higher than that of the PC (p<0.05). Reduction in OXS and trans-amelodentinal diffusion of H_2O_2 occurred when all the BG35%s with MnO_2 were used. The addition of 6 and 10 mg/mL of MnO_2 to BG35% increased the CV in comparison with PC, confirmed by the cell morphology analysis. An increase in FR and $OH^•$ formation was observed when 10 mg/mL of MnO_2 was added to BG35%.

Conclusion: Catalysis of BG35% with MnO_2 minimized the trans-amelodentinal diffusion of H_2O_2 and toxicity of the product to pulp cells. BG35% containing 10 mg/mL of MnO_2 potentiated the decomposition of H_2O_2, enhancing the generation of FR and $OH^•$, as well as the efficacy of the in-office tooth therapy.

INTRODUCTION

The perception of tooth color alteration following dental bleaching therapy is believed to be caused by the interaction of hydrogen peroxide (H_2O_2) and its degradation products with the chromophores present in enamel and dentin.[1-4] However, studies have shown that a large quantity of residual H_2O_2 that does not interact with the chromophores, termed as free-H_2O_2, remains in dentin[5-6], where it can rapidly diffuse to reach the pulp chamber.[7,9-11] Once in contact with pulp cells, the H_2O_2 causes cell injury and pulp necrosis associated with tissue inflammation,[7,8] which has frequently been correlated with post-bleaching (in-office) tooth sensitivity.[6-8,12] Therefore, new dental bleaching protocols have been evaluated with the purpose of preventing these negative side effects caused by this professional esthetic therapy.[13-14]

With the aim of improving the efficacy and simultaneously minimizing the biological damage caused by in-office tooth bleaching, some studies have been developed about incorporating catalysts into gels with high concentrations of H_2O_2.[15-18] The purpose of this alternative is to accelerate the process of H_2O_2 decomposition into free radicals with an extremely short half-life (HLT). This strategy leads such molecules to be rapidly eliminated from the dental tissues after reacting with the chromophores.[1-2,18] Researchers have shown that hydroxyl ($OH^•$, HLT=10^{-9} sec), singlet oxygen (O^{-2}, HLT=10^{-5} sec), and peri-hydroxyl ($HO^{2\cdot}$, HLT=7 sec) are the main free radicals derived from the dissociation of H_2O_2.[19-20] Duque and others[18] demonstrated that the incorporation of catalysts into a bleaching gel with 35% H_2O_2 reduces the toxicity of the product by over 60%. Taking into account these data, some enzymes and salts of transition metals, such as Fe, Cu, Cr, and Mn, may be used as catalysts to promote the dissociation of H_2O_2 into free radicals.[15-18] Therefore, the generation of more reactive molecules with reduced half-life appears to be fundamental for the development of innovative bleaching gels, which can improve the efficacy as well as the compatibility of the in-office bleaching therapy with pulp tissue.

Chemical catalyzers derived from manganese (Mn) have been extensively studied[19-23] because, in addition to their effective catalyzer potential, Mn is abundantly available on the planet,[24] making it a low-cost product.[25-30] Furthermore, the ionic compounds arising from Mn act as co-factors of innumerable enzymes that participate in important biological processes related to the production of energy.[26] Another aspect to be considered is the ample variety of possible groupings to be formed with Mn acting as the central chemical element. This makes it possible to obtain different catalyzers for the formulation of chemical activators.[24,27] It is also imperative to emphasize that Mn-based oxides are capable of catalyzing organic substances since their oxidized form has greater reduction potential than their reduced form.[31-32] Another interesting benefit of chemical catalyzation via metallic oxides, when compared with enzymatic catalyzation, lies in the fact that enzymatic activity, in addition to being high cost, is completely influenced by the pH and temperature of the medium.[33]

The main objective of the present study was to assess the esthetic efficacy, cytotoxicity, and kinetics of

the decomposition of H_2O_2 present in a commercial bleaching gel with 35% H_2O_2, which was chemically activated with manganese oxide (MnO_2). The null hypothesis of this study was that the incorporation of MnO_2 into a highly concentrated bleaching gel does not influence the esthetic efficacy, cytocompatibility, and decomposition of the H_2O_2 present in the dental product.

METHODS AND MATERIALS

SAMPLE PREPARATION

Eighty enamel/dentin discs (DiE/D) were obtained from the buccal surface of bovine incisors by using a diamond trephine bur (Dinser Broca Diamantadas LTDA, São Paulo, SP, Brazil) coupled to a bench drill (FSB 16 Pratika, Schultz, Joinville, SC, Brazil). The disc diameters were standardized to measure 5.6 mm, and the total thickness (enamel/dentin) was established at 2.3 ± 0.2 mm, by wear performed on dentin with water abrasive papers grit 400 and 600 (T469-SF- Norton, Saint-Gobam Abrasivos Ltda, Jundiaí, SP, Brazil), as described by de Oliveira Duque and others.[14]

After regularizing the dentin surface, prophylaxis of the enamel surface was performed with pumice stone and water to eliminate superficial pigments. The dentin was treated with EDTA (ethylenediaminetetraacetic acid; Sigma-Aldrich Corp, St Louis, MO, USA) 0.5 N for 30 seconds to remove the smear layer.[14] Afterward, the discs were placed in a white silicone matrix, in a standardized manner, so that only the enamel surface remained exposed. The spectrophotometer (Color Guide 45/0; BYK-Gardner GmbH, Geretsried, BAV, Germany) was positioned over each specimen with the support provided by the appliance. Then, three initial readouts were taken to obtain a mean of the color values of each sample, determined by the coordinates L*a*b* (CIE L*a*b* system). The values of the coordinates L*a*b* (La Commission Internationale de l'Eclairage [CIE]) were measured with a wavelength ranging from 400 nm to 700 nm, standard illuminant D65, and angle of illumination/observation 45/0°.[14]

The DiE/D discs were submitted to an intrinsic staining protocol after initial color readout, by means of infusion in 2 mL of black tea (Matte Leão, Curitiba, PR, Brazil) for 24 hours.[2-14] After staining, the enamel surface was submitted to prophylaxis and the discs underwent another color readout to determine the mean values of the variables L* (48.61±5.82) and b* (7.07±5.67), because this guide is the standard of tooth staining. Subsequently, the discs with similar mean values of L* and b* were distributed into to the following groups: [NC] - without treatment (Negative Control); [PC] - gel with 35% H_2O_2 (Whiteness HP - WHP, FGM, Joinville, SC, Brazil; Positive Control); [WHP+ 2(MnO_2)] - gel with 35% H_2O_2 containing 2 mg/mL MnO_2; [WHP+6(MnO_2)] - gel with 35% H_2O_2 containing 6 mg/mL MnO_2; and [WHP+10(MnO_2)] - gel with 35% H_2O_2 containing 10 mg/mL MnO_2 (Table 1).

Bleaching Procedure

The chemical activator manganese oxide (MnO_2, Santa Cruz Biotechnology, Texas, USA) was incorporated into the thickener of the commercial bleaching gel (WHP) so that the concentration of the chemical activator would remain at 2 mg/mL, 6 mg/mL, and 10 mg/mL. Then, three drops of the liquid phase containing 35% H_2O_2 (thickener/H_2O_2 proportion in accordance with the manufacturer's indication) were added to the thickener

Table 1: *Experimental Groups and Controls Established According to the Incorporation, or Not, of Different Concentrations of MnO_2 into Bleaching Gel with 35% H_2O_2*

Groups	Treatments	MnO_2 Concentrations	Protocol of Application
NC	Without treatment	—	—
PC	Bleaching Gel (WHP) with 35% H_2O_2	—	Three 15-min applications
WHP+2(MnO_2)	Bleaching Gel (WHP) with 35% H_2O_2+ MnO_2	2 mg/mL	Three 15-min applications
WHP+6(MnO_2)	Bleaching Gel (WHP) with 35% H_2O_2+ MnO_2	6 mg/mL	Three 15-min applications
WHP+10(MnO_2)	Bleaching Gel (WHP) with 35% H_2O_2+ MnO_2	10 mg/mL	Three 15-min applications

Abbreviations: NC, negative control; PC, positive control; WHP, whiteness HP (commercial bleaching gel). "—" signifies that no concentrations of MnO_2 were used. In the negative control group (NC), no protocol of bleaching gel application was used

whether or not it contained MnO_2. After this, 20 μL of the bleaching gels and controls were applied to the enamel of the discs for 15 minutes. This procedure was repeated another two times until a total of 45 minutes of bleaching treatment was concluded. After each application of the bleaching gel, the enamel surface of the discs was rinsed with sterilized water or PBS for bleaching efficacy or cytotoxicity analysis, respectively.

Immediately after obtaining the experimental bleaching gels containing 2 mg/mL, 6 mg/mL, and 10 mg/mL of MnO_2, the homogeneity of the products, and possible formation of bubbles or colored residues during the reaction, were visually analyzed. After this, the variation in pH and temperature was verified by means of a bench microelectrode (HI-2221 Calibration Check pH/Bench Meter; Hanna Instruments Ltd, Leighton Buzzard, AD, United Kingdom). These measurements were taken every 15 minutes in all the bleaching gels, whether or not they contained MnO_2.

Evaluation of Bleaching Efficacy (ΔE and ΔWI)

Forty DiE/Ds (n=8), distributed among the study groups, were stored in an environment with 100% humidity, at 37°C, for 72 hours.[14] Immediately after the bleaching procedures, the DiE/D were again stored in an environment with 100% humidity, at 37°C, for an additional 72 hours. Thus, readouts were performed again to obtain the color change data. Complete color change was obtained by means of the formula $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. The whiteness index for Dentistry was calculated using the L*, a*, b* parameters of transmittance analysis, according to the equation: $\Delta WI = 0.511L - 2.3424a - 1.100b$,[34] in which higher WI values indicate whiter samples and lower WI values indicate darker samples. According to this index, the whiter the material, the higher and more constant the reflectance across the visible wavelength range.[34] The baseline and final (after bleaching) whiteness index was calculated according to: $\Delta\Delta WI = \Delta WI_{\text{after bleaching}} - \Delta WI_{\text{baseline}}$.

Cytotoxicity Evaluation

Cell Culture—Immortalized odontoblast-like MDPC-23 cells, stored in the Experimental Pathology and Biomaterials Laboratory of the Araraquara Dental School - UNESP, SP, Brazil, were used in the study. These cells were seeded in wells of 96- and 24-well plates (KASVI Imp, Curitiba, PR, Brazil) with Dulbecco's Modified Eagle's Medium (DMEM; GIBCO, Grand Island, NY, USA) culture medium, containing 10% Bovine Fetal Serum (BFS; GIBCO, Grand Island, NY, USA), 100 IU/mL of penicillin, 100 μg/mL of streptomycin, and 2 mmol/L of glutamine (GIBCO, Grand Island, NY, USA), in a humidified atmosphere, at 37°C, with 5% of CO_2 and 95% of air.[2,3-14]

Experimental Procedure—Forty DiE/Ds were adapted to artificial pulp chambers (APCs), using two silicone rings for this purpose. After sealing the edges of the discs with baseplate wax (pink) (7 Wax Pink Wilson, Polidental, Cotia, SP, Brazil), the disc/APC sets were sterilized in ethylene oxide (Acecil, Central de Esterilização Comércio e Indústria LTDA, Campinas, SP, Brazil).[2,13-14] Then, the DiE/D/APC sets were positioned in wells of 24-well plates (KASVI Imp) containing 1 mL of DMEM without BFS so that the solution remained in contact with the dentin and exposed enamel, either to receive the treatments, or not (Table 1).[35] Immediately after concluding the bleaching procedures, the extracts (DMEM + gel components that diffused through the DiE/D) were collected, homogenized, and divided into aliquots of 100 μL for the purpose of performing the tests described below.

Cell Viability (MTT Assay)—One aliquot of 100 μL of the extracts was applied to the MDPC-23 cells (n=8) for 1 hour, which had previously been seeded in wells of 96-well plates. Then, the extracts were aspirated and the cells were incubated for 4 hours in contact with 90 μL of DMEM + 10 μL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich Corp, St Louis, MO, USA) in the concentration of 5 mg/mL of PBS, in the ratio of 10:1. After this period, the formazan crystals were dissolved in 100 μL of an acidified isopropyl alcohol (0.04 N HCl) solution, and the absorbance was measured at 570 nm (Synergy H1, Biotek). The mean absorbance value obtained in the NC Group was considered 100% cell viability, and this parameter was used to calculate the cell viability of the other groups.[2,13-14]

Intracellular Reactive Oxygen Species (Oxidative Stress - H_2DCFDA Probe)—The cells seeded in wells of 96-well plates (KASVI Inp) were pretreated with the fluorescent probe carboxy-H_2DCFDA (Invitrogen, San Francisco, CA, USA) in the concentration of 5 μM at 37°C for 30 minutes, and were then exposed to 100 μL of the extracts (n=8). Immediately afterward, fluorescence was evaluated at 492 nm wavelength excitation and 517 nm wavelength emission (Synergy H1, Biotek), and the values were normalized by the mean values of Group NC.[1,2,35]

Analysis of Cell Morphology by Scanning Electron Microscopy (SEM)—For this analysis, the MDPC-23 cells were seeded on glass slide measuring 13 mm in diameter (KASVI Imp), positioned on the bottom of wells of 24-well plates. After being exposed to 100 μL of the extracts (n=4), the cells were fixed in 2.5% glutaraldehyde (Vetec Química Fina LTDA, Duque de Caxias, RJ,

Brazil) for 24 hours and post-fixed in 1% osmium tetroxide (Sigma-Aldrich). After this, dehydration was performed with ascending changes of ethanol solutions (30%, 50%, 70%, 95%, and 100%) and chemical drying with HMDS (1,1,1,3,3,3-hexamethyldisilazane; Sigma-Aldrich). Then, the samples were positioned on metal stubs, stored in a glass desiccator (Labor Quimi, Poá, SP, Brazil) for seven days, at ambient temperature, and sputtered with gold.[1,2,13-15] Finally, the samples were evaluated by SEM (5kv; JEOL JSM 6610, JEOL Ltd, Akishima, Tokyo, Japan) when photomicrographs were obtained at 1000× and 4000× magnification.

H_2O_2 Quantification

To determine the quantity of H_2O_2 that diffused through the enamel/dentin discs, an aliquot of 100 μL of the extracts (n=8) was placed in wells of 24-well plates containing 900 μL of an acetate buffer solution (2 mol/M, pH 4.5). After this, a volume of 500 μL of this solution was transferred to tubes containing 100 μL of Leuco Crystal Violet (LCV) coloring reagent (0.5 mg/mL; Sigma-Aldrich), 50 μL of a horseradish peroxidase (HRP) enzyme solution (1 mg/ml; Sigma-Aldrich), and 2.750 mL distilled water. The absorbance of the solutions was measured in a spectrophotometer at a wavelength of 596 nm (Synergy H1, Biotek). The optical density values were converted into μg H_2O_2 per mL of extract (standard curve), and these values were transformed into percentages, considering the positive control group (35% 3×15) as being 100% of residual H_2O_2 diffusion.[1,2,14,35]

Kinetics of the Decomposition of H_2O_2

For this analysis, the concentration of the chemical activator MnO_2 with the best esthetic and biological performance was selected. The aim of this stage was to evaluate whether the presence of the catalyzer induced the formation of free radical and $OH^{\bullet}$ radicals in the bleaching gel (n=3) during the time interval of 15 minutes. To determine the production of free radicals, an aliquot of the selected bleaching gel was diluted in buffer solution, in the ratio of 1:10 in each time interval of analysis. After this, 50 μL of this sample was transferred to wells of 96-well plates. To proceed with quantification, the solutions were incubated at 37°C with the carboxy-H_2DCFDA probe (Invitrogen, Eugene, OR, USA) in the concentration of 1 mM (1:1), and the emission of fluorescence was evaluated with excitation of 492 nm and emission of 527 nm (Synergy H1, Biotek) for 15 minutes. To quantify the $OH^{\bullet}$ released by the selected bleaching gel, the fluorescence probe of the OxiSelect Hydroxyl Radical Antioxidant Capacity (HORAC) Activity Assay (Cell Biolabs Inc, San Diego, CA, USA) kit was used, of which the principle is based on oxidation of the probe by $OH^{\bullet}$ via transference of an atom of oxygen. Thus, the fluorescence of the reaction is reduced in the presence of $OH^{\bullet}$. For this analysis, 20 μL of the samples were incubated with 140 μL of the probe, and the fluorescence was monitored at 480 nm excitation and 530 nm emission (Synergy H1, Biotek) throughout the period of 45 minutes of incubation at ambient temperature.[1,2]

Statistical Analysis

The quantitative data were evaluated relative to the normal adherence curve and all showed normality (Shapiro-Wilk, $p>0.05$) and homogeneity of variance (Levene, $p>0.05$). Therefore, these data were applied to the one-way analysis of variance tests that were complemented with the Tukey test for color and cytotoxicity assays. For the analysis of the kinetics of the decomposition of H_2O_2, the Student's-*t* test was applied. All statistical analyses inferences were based on the level of significance of 5%. The statistical power of the analyses was calculated by means of DDS Research (Statistical Power Calculator, α=5%). Visual analyses were performed for the qualitative data.

RESULTS

Analysis of pH and Temperature of MnO_2-Containing Bleaching Gels

Visual analysis of the bleaching gels allowed for the determination of the fact that there was no formation or precipitation of residues after the incorporation of MnO_2. The possible changes in pH and temperature were evaluated in the time intervals of 0.5, 5, 10, and 15 minutes after addition of the chemical activator to the commercial bleaching gel (WHP). The pH of the gels activated with MnO_2 were maintained close to neutral or slightly alkaline (Figure 1A). The temperature also remained stable during all periods, and did not exceed 30°C (Figure 1B).

Bleaching Efficacy (ΔE and ΔWI)

All the bleached groups showed a significant increase in the values of ΔE when compared with NC Group. Significant increases of 15% and 31% in esthetic efficacy occurred after addition of 6 mg/mL and 10 mg/mL of the catalyzer MnO_2, respectively, to the gel, in comparison with PC Group. The bleaching gel with 10 mg/mL of MnO_2 showed the highest ΔE values when compared with the other groups (Figure 2A). All the bleached groups showed a significant increase in the values of ΔWI when compared with the NC Group. No

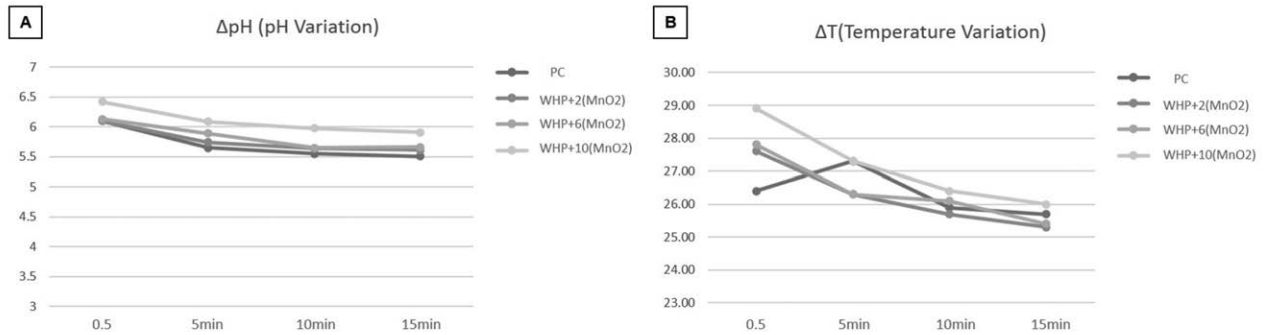

Figure 1. *(A): Analysis of variation in pH. Comparative graph of variation in pH (ΔpH) between WHP and bleaching gels catalyzed with MnO_2, represented by Groups PC, WHP+2(MnO_2), WHP+6(MnO_2), and WHP+10(MnO_2). The analyses were performed in time intervals of 0.5, 5, 10, and 15 minutes. (B): Analysis of variation in temperature. Comparative graph of variation in temperature (ΔT) between WHP and bleaching gels catalyzed with MnO_2, represented by Groups PC, WHP+2(MnO_2), WHP+6(MnO_2), and WHP+10(MnO_2). The analyses were performed in time intervals of 0.5, 5, 10, and 15 minutes.*

differences were found in the whiteness index among groups after the addition of 2 mg/mL and 6 mg/mL when compared with PC Group. The addition of 10 mg/mL showed the highest ΔWI values in comparison with the other groups (Figure 2B).

Cytotoxicity

The NC Group was considered to have a cell viability of 100%. Reduction in cell viability and increase in oxidative stress occurred in all the bleached groups ($p<0.05$). Groups WHP+6(MnO_2) and WHP+10(MnO_2) showed 28% and 45% higher cell viability, respectively, in comparison with PC Group ($p<0.05$; Figure 3A). All the groups in which bleaching was performed with gel containing MnO_2 showed significant reduction in oxidative stress when compared with PC Group (Figure 3B), with outstanding results for Group WHP+10(MnO_2). Changes in number and morphology of cells adhered to the substrate were observed by SEM in PC Group in comparison with NC Group. However, a less deleterious effect on cells occurred in the groups in which 6 mg/mL and 10 mg/mL of MnO_2 were added to the bleaching gel, and the cells of Group WHP+10(MnO_2) exhibited a morphological pattern similar to that of the NC Group (Figure 4).

Quantification of Residual H_2O_2

Significant reduction in the diffusion of residual H_2O_2 occurred in all the groups in which bleaching was performed with gel containing MnO_2, in comparison with the PC Group (Figure 3C). Group WHP+10(MnO_2) had a lower quantity of residual H_2O_2

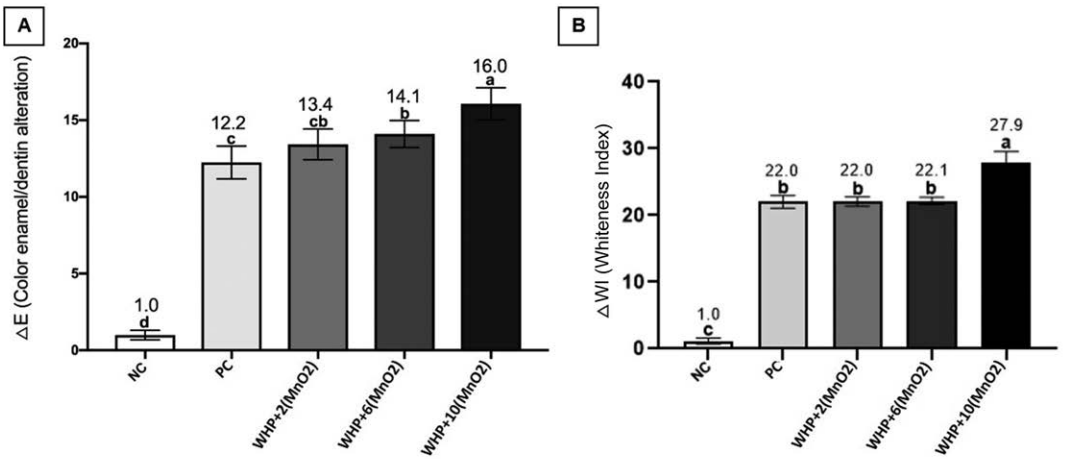

Figure 2. *(A): Analysis of bleaching efficacy (ΔE) of bleaching gels. Bar graph of mean values and standard deviation determined by calculation of total ΔE of bleached groups. (B): Analysis of bleaching efficacy (ΔWI) of bleaching gels. Bar graph of mean values and standard deviation determined by calculation of total ΔE of bleached groups. Different letters demonstrate statistically significant difference among groups (One-way ANOVA; Tukey Test, $\alpha=0.05$ / n=8)*

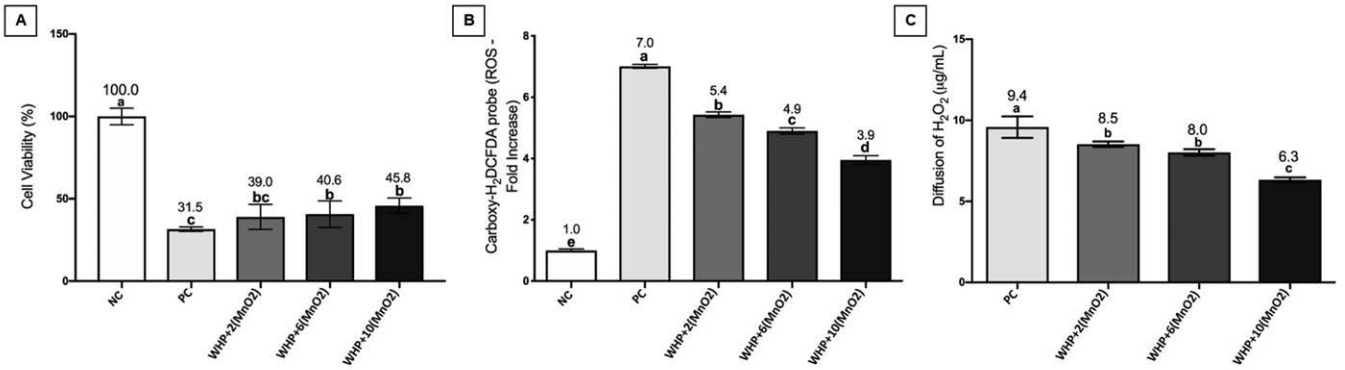

Figure 3. *(A): Analysis of cell viability performed by MTT assay. Bar graph showing mean values and standard deviation of cell viability. (B): Analysis of cell oxidative stress measured by the OXS values present in the culture medium. Bar graph of mean values and standard deviation of emission of fluorescence normalized by control of carboxy-H_2DCFDA probe. (C): Analysis of residual H_2O_2: bar graph of mean values and standard deviation of H_2O_2 concentration in the extracts. Different letters demonstrated statistically significant difference among groups. (One-way ANOVA; Tukey Test, α=0.05 / n=8)*

in the culture medium when compared with the other bleached groups.

Kinetics of the Decomposition of H_2O_2

Because of having shown the best results of esthetic efficacy and reduction in cytotoxicity, only the bleaching gel containing 10 mg/mL of the catalyzer MnO_2 was evaluated. A significant increase in the production of free radicals (Figure 5A) and hydroxyl radicals (Figure 5B) was observed in this Group WHP+10(MnO_2) in comparison with the PC Group, and the formation of free radicals reached its peak in the time interval of 15 minutes.

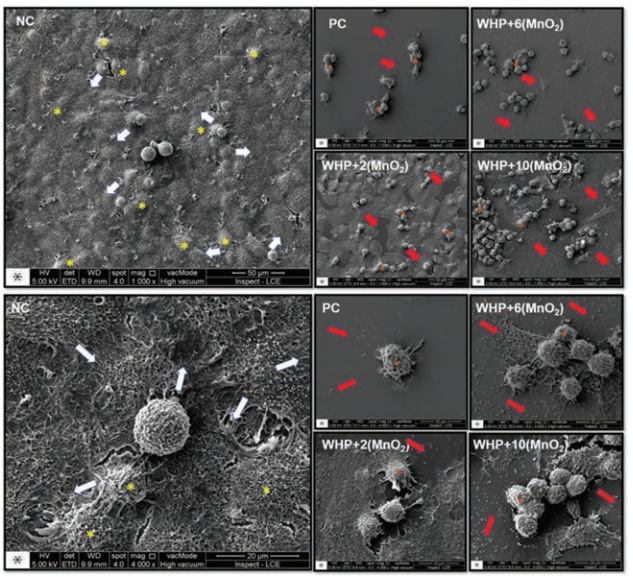

Figure 4. *Representative images obtained by scanning electron microscopy (SEM) for each study group. It was possible to observe a significant reduction in the quantity of cells adhered to the glass slide in the NC Group, which had a well-defined polygonal morphology (*) with cytoplasmic projections (white arrows), whereas in the bleached groups it was possible to observe the bottom of the glass slide onto which the cells exposed to the extract detached themselves (red arrows). Furthermore, it was possible to observe morphological change in the cell surface after bleaching (*). Magnifications of 1000× and 4000× (n=4).*

DISCUSSION

The chemical decomposition of H_2O_2 in an aqueous medium, which may be potentiated by different factors—such as those described by Choudhary and others[36]—appears to be an interesting alternative for making in-office tooth bleaching more compatible with the dentin-pulp complex, without harming the esthetic outcome of the treatment.[1-2,18] Suty and others[19] reported that catalyzers could accelerate the decomposition of H_2O_2, which resulted in the formation of intermediate chemical species. The dissociation of this reactive oxygen species (ROS), which is the main active component of bleaching gels, into other highly reactive molecules with a short half-life has been considered crucial for reducing the toxicity of this modality of esthetic therapy, widely used in dental offices.[2] Therefore, with the aim of increasing the reactivity of bleaching gel with the dental structures and minimizing the trans-amelodentinal diffusion of the toxic components released by the product, in the present study the proposal was to incorporate the chemical activator manganese oxide (MnO_2) into a commercial bleaching gel containing 35% H_2O_2.

Initially, 2 mg/mL, 6 mg/mL, and 10 mg/mL of MnO_2 were incorporated into the thickener, and the new products obtained remained viscous, with a homogeneous pattern, without forming colored residues. These bleaching gels containing MnO_2 showed stability of pH (close to neutral) and temperature during the different time intervals of

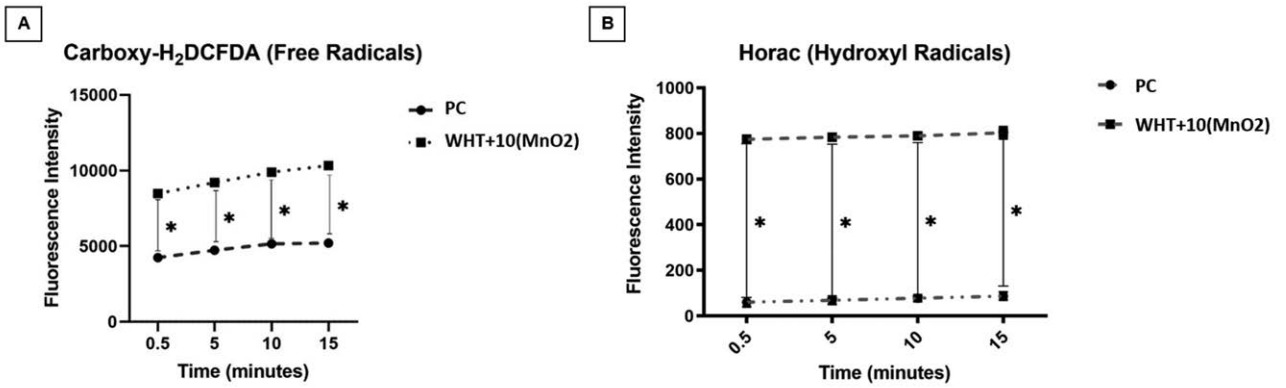

Figure 5. *(A): Analysis of the kinetics of decomposition of H_2O_2 — formation of free radicals (carboxy-H_2DCFDA probe). The values corresponded to the emission of fluorescence in the two groups evaluated and tested in the time interval of 15 minutes (0.5, 5, 10, and 15 minutes). (B): Analysis of the kinetics of decomposition of H_2O_2 — formation of hydroxyl radicals (HORAC probe). The values corresponded to the emission of fluorescence in the two groups evaluated and tested in the time interval of 15 minutes (0.5, 5, 10, and 15 minutes). The symbol * indicated statistical difference between the groups in the formation of hydroxyl radicals within each time interval in the analysis (Student's-t Test; n=3).*

evaluation. In a recent study, Guan and others[37] used amperometry to demonstrate that the catalytic current of chemical activation performed by MnO_2 attains the ideal point at a pH within a range of values between 5.0 and 7.0, at the time when the chemical interaction of this activator with H_2O_2 reaches its maximum point. In addition, bleaching agents with neutral pH do not cause changes in the enamel surface that may favor the inward diffusion of H_2O_2.[38] Therefore, in the present research the stability pH of the bleaching gels containing MnO_2 (close to 7.0) certainly favored the kinetics of decomposition of H_2O_2, potentiating the generation of free radicals (Figure 5A) and hydroxyl radicals (Figure 5B), which was clearly observed in Group WHP + 10 (MnO_2).

This positive behavioral profile of the bleaching gels containing MnO_2 enabled continuity of the subsequent analyses, which consisted of determining the esthetic efficacy and cytotoxic potential of these new products. With regard to the esthetic efficacy, a significant increase in the total ΔE of the DiE/D was observed with the use of the bleaching gel containing 6 mg/mL of MnO_2. Nevertheless, the highest ΔE values were obtained in Group WHP+10(MnO_2), in which the total change in color was 31% higher in comparison with PC Group, which in this study, represented the conventional in-office tooth bleaching protocol. This result could be explained, even partially, by the excellent catalytic activity that transition metals, such as MnO_2, have when they come into contact with substances with oxidative potential (H_2O_2), and this activity is further enhanced when the medium contains a high concentration of H_2O_2.[36] Therefore, one may suggest that the greater availability of MnO_2 in the bleaching gel containing 10 mg/mL of this chemical activator may have accelerated the decomposition of H_2O_2. One of the possible interpretations for the mechanism of this reaction consists of the process of reduction of MnO_2 that occurs in a medium rich in H_2O_2:[20]

$$MnO_2 + H_2O_2 \rightarrow MnO \text{ (or } Mn_2O_3) + O_2 + H_2O$$

$$MnO \text{ (or } Mn_2O_3) \rightarrow MnO_2 + 2e^-$$

Concerning the trans-amelodentinal cytotoxicity of the products evaluated in this study, reduction in intracellular oxidative stress values of approximately 23%, 30%, and 44% occurred when 2 mg/mL, 6 mg/mL, and 10 mg/mL of MnO_2, respectively, were incorporated into the bleaching gel. This datum is directly related to the significant reduction in toxic residual H_2O_2 present in the culture medium, as was demonstrated by means of the leucocrystal violet test. Values of 28% and 45% higher cell viability in comparison with the PC Group were observed in Groups WHP+6(MnO_2) and WHP+10(MnO_2), respectively. These data were corroborated by the qualitative analysis of MDPC-23 cells by SEM, in which it was observed that the higher the concentration of MnO_2 incorporated into the gel, the larger the number of cells that remained adhered to the substrate. Furthermore, the cells of Group WHP+10(MnO_2) were shown to have a morphology similar to that of the cells of the NC Group, which showed evidence of the reduction in the toxic potential of the bleaching gel containing MnO_2. Recently, Ortecho-Zuta and others[2] demonstrated that a bleaching gel with 35% H_2O_2 activated with horseradish

peroxidase (HRP) enzyme resulted in cell viability that was almost two times higher than that observed for the same gel without the addition of the enzyme. However, the authors reported that in spite of the limited trans-amelodentinal toxic effect of the gel with HRP, the cells that remained adhered to the substrate exhibited important morphological changes, characterized by the reduction in their size, rupture of the membrane, and/or loss or contraction of the cytoplasmic processes. This cellular response may have been triggered by oxidative stress, which causes direct cellular lesions or even reduces the repair capacity of cells in the long term.[14] Studies have shown that pathological oxidative stress in pulp cells causes acute tissue inflammation[39-40] with a consequent expression of proteases and degradation of the extracellular matrix,[41] which may result in areas of pulp necrosis.[7,8] In the present investigation, it was observed that the addition of 10 mg/mL MnO_2 to the bleaching gel reduced by 67% the quantity of H_2O_2 that diffused through the enamel and dentin. In a recent study, using an *in vitro* experimental model similar to that used in the present research, the authors demonstrated that concentrations of H_2O_2 ranging from 2.55 μg/mL to 3.54 μg/ mL were sufficient to reduce pulp cell viability by 31.5% to 61.7%.[42] Therefore, in the present study it was to be expected that the concentration of 6.3 μg/mL of H_2O_2 that diffused through the enamel/dentin discs would also cause some toxicity to the MDPC-23 cells in culture, however, with a lower intensity than that observed for the PC Group.

In the present study, the reduction in the concentration of residual H_2O_2 in the culture medium was associated with the chemical catalyzation of H_2O_2 mediated by MnO_2. This result, which was more evident in Group WHP+10(MnO_2), can be justified by greater availability of the catalyst present in the bleaching gel. Based on the fact that manganese is a transition metal, one may suggest that the mineral phase of this oxide could have catalyzed the H_2O_2 by means of a Fenton-like reaction,[43] which explains the high generation of $OH^{\bullet}$. However, it is necessary to emphasize that the degradation rate of organic pigments increases until achieving the ideal concentration of the catalyst. It is known that concentrations beyond this threshold do not enhance the degradation levels of such pigments. This is because the high concentrated free radicals tend to react with each other, then being quickly consumed.[45-46] Another important aspect related to the high catalytic potential of MnO_2 is that the metallic portion of this oxide tends to react quickly with H_2O_2 at neutral/basic pH.[47-48] These properties and characteristics of MnO_2 may have enhanced the degradation of the bleaching gels used in this study.

It is known that high tissue levels of manganese in the central nervous system creates clinical symptoms of cognitive dysfunction, behavioral changes, and movement disorders resembling Parkinson's disease.[49] However, Finley and others[50] followed women who were fed with 0.8 or 20 mg Mn/day for 60 days to determine how the diets containing low or high amounts of Mn affect neuropsychological measures and basic metabolic function. The authors showed that there was no association between Mn intake and performance in neurological tests. They concluded that efficient mechanisms operate to maintain Mn homeostasis over a wide range of Mn intake levels that can be found in the diet and that, for healthy adults, Mn intake from 0.8 to 20 mg for 8 weeks does not result in Mn toxicity. In the present study, concentrations of MnO_2 lower than 10 mg/mL was added to a commercial bleaching gel, which has been recommended to be applied for a short time (45 minutes) on enamel. Therefore, it is not expected that the addition of such oxide into the dental product causes any neurotoxicity risk to humans. So, according to the interesting scientific data achieved in the present research, it is possible to consider MnO_2 a promising catalyzer to act as coadjutant in the chemical catalysis of H_2O_2. The presence of the catalyst was responsible for the reduction of the intracellular oxidative stress and diffusion of H_2O_2. These analyses directly impact on the reduction of toxic effects caused by the gels with 35% H_2O_2. In addition, it was possible to observe a significant increase in the esthetic result achieved with in-office bleaching. This increase is mainly related to free radical production due to high H_2O_2 degradation in the presence of the catalyst. Based on these data, the null hypothesis of this study was rejected.

Despite the motivating results presented, it is important to consider that *in vitro* data, such as these obtained in the present study, cannot be immediately extrapolated to clinical situations.[38] This is because the outward dentin fluid movement, presence of collagen, cytoplasmic processes of the odontoblasts, and other structures within the dentinal tubules of vital teeth may interfere in the trans-amelodentinal diffusion of H_2O_2 and, therefore, in the cytotoxicity of the bleaching gels. Based on the results of the present laboratory research, one may suggest that new *in vitro* studies must be conducted to determine the dynamics of MnO_2 decomposition, such as the ideal concentration of this chemical catalyzer being incorporated into bleaching gels so that even more effective products may be obtained, with potential to be evaluated *in vivo* and perhaps be more safely used clinically, without causing discomfort to patients.

CONCLUSION

According to the methodology used in the present research, it was concluded that the activation of the bleaching gel with 35% H_2O_2, by means of incorporating 10 mg/mL of MnO_2 into the product, accelerated the decomposition of H_2O_2, stimulating the generation of $OH^{\bullet}$ and other free radicals. These effects of MnO_2 increased the esthetic efficacy of the in-office tooth bleaching and led to a smaller quantity of residual H_2O_2 being diffused through enamel and dentin, thereby minimizing the trans-amelodentinal toxic effect caused to pulp cells by this professional therapy.

Acknowledgments

This work was supported by São Paulo Research Foundation/ FAPESP [Grants number: 2017/22739-9 and 2018/14457-6] and National Council for Scientific and Technological Development / CNPq [Grants number: 302047/2019-0 and 408721/2018-9].

Conflict of Interest

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

(Accepted 10 September 2021)

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Quality of Cure in Depth of Commercially Available Bulk-fill Composites: A Layer-by-layer Mechanical and Biological Evaluation

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Clinical Relevance

Of the nine bulk-fill materials studied, only four conform to the "bulk" designation, ie, they show no significant difference up to a depth of 4 mm for all properties considered. Among these four, very large differences could be observed.

SUMMARY

Despite their popularity, the use of bulk-fill composites remains controversial, both in terms of their properties and their in-depth development. The objectives of the present work were (1) to provide a more comprehensive evaluation of the quality of cure in depth of commercially available bulk-fill composites by combining various key mechanical and biological characterization methods, (2) to evaluate the inter-material differences when optimally cured, and (3) to evaluate the efficiency of an antioxidant—N-acetyl-cysteine (NAC)—to restrain the adverse effects of the leached components on cell viability.

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http://doi.org/10.2341/21-084-L

Nine bulk-fill composites (including flowable and high-viscosity materials) were investigated and compared to two conventional resin-based composites, one flowable and one high-viscosity restorative material. The materials were injected or packed into Teflon molds of various configurations, up to 6 mm material thickness. They were then light-cured from the top for 20 seconds with Bluephase G2 (Ivoclar Vivadent, irradiance = 1050 mW/cm^2). The following physico-mechanical properties were measured for the upper (0-2 mm), intermediate (2-4 mm), and lower (4-6 mm) layers: degree of conversion using Raman Spectrometry (DC, in %), microhardness using a Vickers micro-indenter before (VHN dry) and after 24 hours of storage in ethanol (VHN EtOH), and flexural strength (in MPa) and flexural modulus (in GPa) using a three-point bend test. Each composite layer and an uncured layer were also stored for one week in a standard cell growth medium to generate conditioned media. Human dental pulp cells were then cultured for 24 hours with the latter and cell viability was measured using an MTS assay. A similar experiment was repeated with conditioned media produced in contact with uncured composites, with and without the addition of 4 mM NAC. The data were subjected to a Shapiro-Wilk test, then one-way ANOVA or Kruskal-Wallis test, followed either by Tukey's test (inter-material comparison) or by Dunnett's or Dunn's test (comparison between layers relative to the upper one). The level of statistical significance was set at 0.05.

Some materials (EverX, X-traF, VenusBF, X-traB) did not show any significant differences (p>0.05) for any of the properties considered between the intermediate layers compared to the upper one (considered as reference). Others displayed significant differences, at least for some properties, highlighting the value of combining various key mechanical and biological characterization methods when investigating the quality of cure in depth. Significant inter-material differences (p<0.05) were observed when comparing the properties of their upper layer, considered as "optimally" polymerized. Hence, one needs to consider the absolute property values, not only their relative evolution concerning layer thickness. Finally, the use of NAC appeared as beneficial to reduce the risk of harmful effects to dental pulp cells, especially in case of excessive thickness use, and may therefore be of potential interest as an additive to composites in the future.

INTRODUCTION

The indications regarding the use of resin-based composites (RBCs) have considerably evolved over time since their introduction to the market. They were initially used for small restorations, but are now routinely used for larger and larger restorations. However, an increase in the failure rate has also been reported as the number of restored surfaces increases,[1-4] making the mechanical and biological performance of the materials all the more important in large cavities. Due to the need to place the composite restorations in layers,[5,6] the restoration of large cavities with RBCs is very time-consuming for both clinicians and patients. To reduce the procedure duration, two different strategies were developed: (1) changing the photoinitiator to significantly reduce the curing time (below 5 seconds) while maintaining, or in some cases improving, mechanical and biological key material properties[7-10]; and (2) to increase the depth of cure by modifying certain material characteristics, thereby giving birth to a "new" RBC category, namely bulk-fill composites. Most notably, increased light transmission through the composite was obtained by changes in material composition, mainly a reduction in filler content, an adjustment of filler size relative to the light wavelength, and an adaptation of the refractive index between the inorganic and organic fractions.[11]

Bulk-fill composites are marketed as a solution to restore large tissue losses in layers of 4-mm thickness or sometimes more (Table 1). Very few randomized clinical studies comparing the success of bulk-fill composite restorations to conventional ones are currently available, and those available have relatively short follow-up and include restorations of limited size.[12,13] The randomized clinical trial with the longest follow-up period[14] compared a bulk-fill strategy (4-mm bulk-fill RBC covered with 2-mm conventional RBC) to a classic incremental filling, in class I and II cavities. Over the six-year evaluation period, no significant difference could be observed between both groups. Despite being promising, these results need to be verified for other materials, given the large differences in physico-mechanical properties reported within the bulk-fill RBC category.[15] Moreover, the performance of bulk-fill restorations in larger cavities remains unknown and is therefore subject to caution.

One particularly important aspect regarding the performance of RBCs placed in thick layers is their quality of cure in depth. Several methods have been proposed in

Table 1: *List of Tested Materials*

Materials	Abbreviation	Manufacturer	Composite Type	Shade	Batch	Maximum Layer Thickness Recommended by the Manufacturer in Instructions for Use
Grandio	Grandio	Voco (Cuxhaven, Germany)	Hybrid paste conventional composite	A3	1408240	2 mm
Grandio Flow	GrandioF	Voco (Cuxhaven, Germany)	Hybrid flowable conventional composite	A3	1208317	2 mm
Sonic Fill	SonicF	Kerr (Orange, CA, USA)	Bulk-fill paste composite with sonic hand-piece	A3	5139879	5 mm
Tetric Evo Ceram Bulk Fill	TECBF	Ivoclar Vivadent (Schaan, Liechtenstein)	Bulk-fill paste composite	Bulk IVA	540860	4 mm
Venus Bulk Fill	VenusBF	Heraeus Kulzer (Hana, Germany)	Bulk-fill flowable composite	Universal	10105	4 mm
Filtek Bulk Fill	FiltekBF	3M-ESPE (St Paul, MN, USA)	Bulk-fill flowable composite	A3	536127	4 mm
X-tra fil	X-traF	Voco (Cuxhaven, Germany)	Bulk-fill paste composite	Universal	1343523	4 mm
X-tra base	X-traB	Voco (Cuxhaven, Germany)	Bulk-fill flowable composite	Universal	1345335	4 mm
Surefil SDR Flow	SDR	Dentsply (Konstanz, Germany	Bulk-fill flowable composite	Universal	1407000667	4 mm
Ever-X posterior	EverX	GC Europe (Leuven, Belgium)	Bulk-fill paste composite with glass microfibers	/	1309091	4 mm
Fill-up!	Fill-up	Coltene Whaledent (Alstätten, Switzerland)	Dual-cure bulk-fill flowable composite	Universal	F33233	Arbitrary thickness owing to its dual curing properties

the past to evaluate the "depth of cure",[16-18] corresponding to the depth at which the RBC is considered "adequately" cured. It was underlined that the depth of cure values vary greatly depending on the method considered, which may result in an overestimation of the true value.[19] Therefore, a combination of various methods is more likely to provide a more complete assessment of the quality of cure in depth.[16] In this sense, while physico-mechanical properties are often studied in this context, biological aspects are more often omitted or studied separately. Therefore, they need to be integrated into the laboratory evaluation of the performance of thick RBC restorations. Notably, RBCs can release various active compounds such as monomers or photoinitiators,[20]

which can diffuse through the adhesive layer and dentin[21] and ultimately reach pulp cells. Numerous undesirable biological responses have been described following contact of the released substances with the cells,[22] responses that have been so far mostly attributed to an oxidative stress through the generation of reactive oxygen species.[23]

Hence, the objectives of the present work were (1) to provide a more comprehensive evaluation of the quality of cure in depth of commercially available bulk-fill composites by combining various key mechanical and biological characterization methods, (2) to evaluate the inter-material differences when appropriately cured, and (3) to evaluate the efficiency of an antioxidant—N-acetyl-cysteine (NAC)—to restrain the adverse effects of the leached components on cell viability when the composite was unpolymerized, to simulate an inappropriate use.

METHODS AND MATERIALS

Nine bulk-fill RBCs (including flowable and high-viscosity materials) were investigated and compared to two conventional RBCs, one flowable and one high-viscosity restorative material (Table 1).

Mechanical Evaluation

For the evaluation of flexural properties (modulus and strength) in depth, the composites were placed into three rectangular white Teflon molds of 2 mm thickness, 2 mm width, and 25 mm length (Figure 1a). The three Teflon molds were aligned and assembled by two screws, each composite layer being isolated from the others by polyester films to allow ulterior individual processing of each separate layer. The uppermost surface was covered with a polyester film to prevent the formation of an oxygen inhibition layer. Photopolymerization of the three layers was initiated by three successive and non-overlapping irradiations of 20 seconds on the upper side, with the light tip in close contact with the polyester film (inner tip diameter = 9 mm). All light-curing procedures were performed with the Bluephase G2 light-curing unit (Ivoclar Vivadent, Schaan, Liechtenstein) set to "high-power" mode (irradiance = 1050 mW/cm^2 as measured before each experiment by Bluephase Meter - Ivoclar Vivadent, Schaan, Liechtenstein).

The three 2-mm-thick layers—0-2 mm (upper layer), 2-4 mm (intermediate layer), and 4-6 mm (lower layer)—were removed from the molds and polished using SiC paper grit 1000 then placed in distilled water in the dark for one week at 37°C. They were then submitted to a three-point bend test in a universal testing machine (LRX Plus, Lloyd Instruments, Largo, FL, USA) at a crosshead speed rate of 0.75 mm/min until a fracture occurred (n=5). Flexural modulus (based on the tangent to the initial slope) and strength were calculated based on ISO 4049:2000.

For the measurement of microhardness and degree of conversion, the composites were injected into a rectangular white Teflon mold of 5 x 5 mm aperture and 10-mm depth (Figure 1b), covered by a polyester film, and light-cured from the aperture in a single

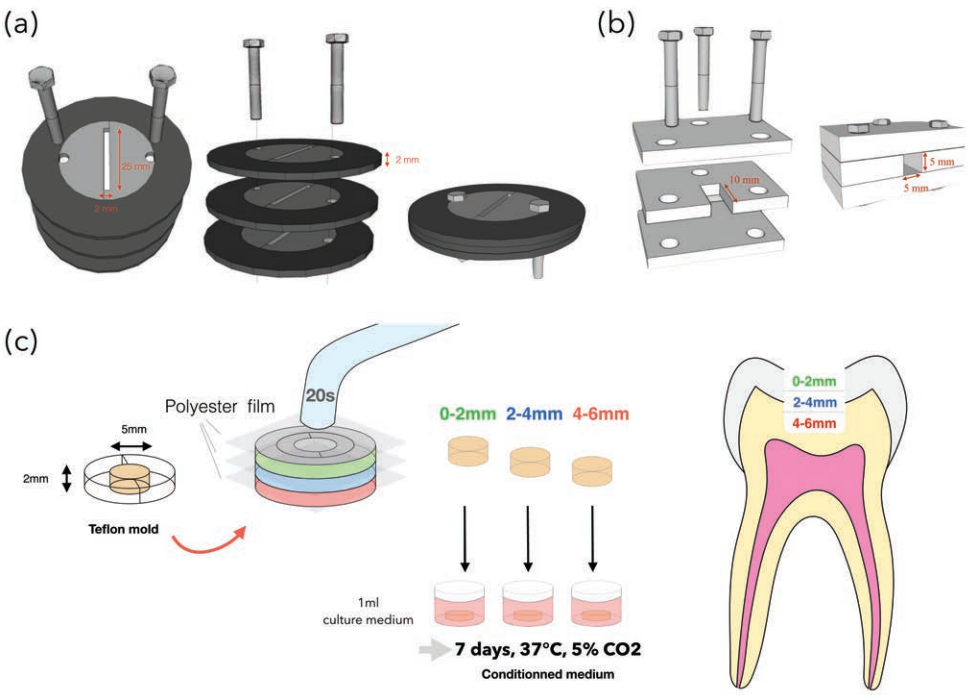

Figure 1. *Experimental setups for layer-by-layer polymerization: (a) molds for samples for 3-point-bending; (b) molds for samples for DC and VHN measurements; and (c) measurement of cell toxicity. (c) presents how each layer of the composite cylinder was then used to produce the conditioned medium.*

20-second irradiation with the same parameters described above. The degree of conversion (DC, in %; n=3) was measured on the side of the sample at 0-, 2-, 4-, and 6-mm depth using a Raman spectrometer (DXR Raman microscope, Thermo Scientific, Madison, WI, USA). Briefly, the samples were excited at 780 nm by a frequency-stabilized single-mode diode laser through a microscope objective (50×) and spectra were obtained in the region 1600 cm^{-1}, with the following conditions: microhole: 50; irradiation time: 60 seconds; number of accumulations: 5; and grating: 400 lines/mm. The DC was then calculated based on the decrease in intensity of the peak corresponding to the methacrylate C=C groups at 1640 cm^{-1} compared to the uncured sample; the aromatic peak at 1610 cm^{-1} was used as the internal standard.[24] On the same samples, Vickers microhardness was measured in dry conditions (VHN dry; n=3) along the side of the sample at a similar depth (0, 2, 4, and 6 mm) using a Durimet microhardness tester (Leitz, Wetzlar, Germany) and applying a 200 g load for 30 seconds. The samples were then immersed in pure ethanol for 24 hours, before re-measuring the microhardness (VHN EtOH; n=3).[15] To enable comparison with flexural properties, an average value was calculated between 0 and 2 mm (upper layer), 2 and 4 mm (intermediate layer), and 4 and 6 mm (lower layer).

Cell Culture

The human dental pulp cells (hDPCs) used in the present work were obtained from the pulps of four wisdom teeth extracted for orthodontic reasons (female patient, 18-years-old) after informed consent. hDPCs were isolated by the outgrowth method.[25] Briefly, after surface disinfection, the teeth were split to recover the pulp tissue, which was then rinsed in a growth medium (see above), minced into small pieces, and placed in 6-well plates. The growth medium was changed every 2-3 days. After 10 days, the cells were harvested using Acutase (Life Technologies, Gent, Belgium), centrifuged, and replated in a new flask. For the next passages and experiments, the cells were harvested upon reaching 80% confluence, and either frozen or plated at 2.5 x 10^5 cells/T75 flask for subsequent use. For all experiments performed in the present work, the cells were used at passage 6.

Biological Evaluation

For the study of hDPC viability, a conditioned medium was produced by incubating composite disks into a cell culture medium (Dulbecco's Modified Eagle Medium—supplemented with 10% bovine serum, L-Glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin, Thermo Fischer-Scientific, Waltham, MA, USA). The composites were packed into three superimposed and aligned white Teflon molds (5-mm diameter, 2-mm thickness) separated by polyester films to allow for individual processing of each separate layer (Figure 1c). Like for the other measurements, the curing light tip was placed in close contact with the polyester film covering the uppermost surface. Each of the three 2-mm-thick disks—0-2 mm (upper layer), 2-4 mm (intermediate layer), 4-6 mm (lower layer)—was then placed in 1 mL of culture medium in a 24-well plate and incubated for 7 days at 5% CO_2, 95% humidity (Figure 1c). After one week, conditioned media were collected and incubated with hDPCs to evaluate their impact on cell viability. Positive controls were obtained by incubating an identical volume of uncured materials. For the flowable materials, a volume of 40 mm^3 corresponding to the volume of the mold was injected directly into the well.

To evaluate the effect of the components released by the materials on cell viability, hDPCs were seeded onto 96-well plates at 10^4 cells per well ($3.03*10^4$ cell/cm^2). The culture was maintained with 5% CO_2, at 37°C for 24 hours to allow cell adhesion. After that, the cell culture medium was removed and replaced by the conditioned medium for 24 hours at 5% CO_2, 37°C, standard culture medium was used as control. Cell viability was assessed using an MTS assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) as per supplier instructions. Briefly, the culture medium was removed from the wells, which were washed three times with PBS. MTS solution (100 µL) was then added to each well for 30 minutes at 5% CO_2, 37°C. The absorbance of each well was then determined using a microplate reader (SpectraMax M2, Molecular devices) at a wavelength of 490 nm.

In a second series of experiments, an antioxidant (N-acetyl-cysteine—NAC) was added to the conditioned media obtained with the uncured materials, to determine whether the cytotoxic effect of the released compounds could be attenuated or annihilated. NAC was prepared as 1 mol/L stock solution in HBSS solution and pH was adjusted to 7.2. In preliminary experiments using various concentrations (0 - 2 - 4 - 6 - 8 - 10 mM), cell viability was affected above 6 mM after 24 hours of incubation (data not shown). The final concentration of NAC added to the medium was therefore 4 mM (n=4 wells per condition).

Statistical Analyses

Statistical analyses were performed using JMP software (SAS Institute, Cary, NC, USA). The normality of the

distributions was verified using a Shapiro-Wilk test, after logarithmic transformation of the data if necessary. For inter-materials comparisons (upper layer), one-way ANOVA was performed followed by Tukey's test for multiple comparisons. For the effect of the layer (intra-material comparisons), one-way ANOVA was performed in case of normal distribution of the data, followed by Dunnett's test for comparison with the layer considered as reference, ie, the upper layer (0-2 mm). When normality was rejected, a Kruskal-Wallis test was performed, followed by Dunn's test for comparison with the reference layer (0-2 mm). The level of statistical significance was set at 0.05.

RESULTS

Mechanical Evaluation

The evolution in depth (upper, intermediate, and lower layers) of the various material properties appeared to be clearly material-related. Indeed, while some materials (EverX, X-traF, VenusBF, X-traB) did not show any significant differences ($p>0.05$) for any of the properties considered between the lower or intermediate layers compared to the upper one (considered as reference), others displayed significant differences, at least for some properties. For the vast majority of the materials and properties, no significant differences could be observed between the upper and the intermediate layer, with no clear trend appearing when comparing bulk-fill or conventional materials. On the contrary, significant differences were frequently detected when considering the lower layer, and this was systematically for the two conventional materials (Grandio and GrandioF) for all properties (Figure 2).

Significant differences ($p<0.05$) in absolute values were observed between the materials for all properties (upper layers), particularly for microhardness and flexural modulus (Table 2). Specifically, with regard to hDPC viability, and in the experimental conditions used, the conditioned media from each composite cured under optimal conditions (upper layer) led to significant inter-material differences in hDPC viability ($p<0.05$; Table 2), with values ranging from 54% (FiltekBF) to 98.7% (GrandioF).

Biological Evaluation

Relative hDPC viability was not significantly different ($p>0.05$) between intermediate and upper layers for all materials, except for GrandioF for which a slight increase was observed. On the contrary, the lower composite layer led to a reduction of hDPC viability compared to the upper layer, in a significant manner ($p<0.05$) for half of the materials investigated.

A significant reduction in relative cell viability was observed for cells grown in a conditioned medium prepared with uncured composites compared to the lower composite layer ($p<0.05$ for all materials except SonicF, TECBF, and VenusBF). Average relative values for uncured composites ranged from 0 to 60.3% compared to the standard growth medium (Figure 3). The addition of 4 mM of NAC to the conditioned media resulted in a significant increase in relative cell viability for all materials ($p<0.05$), with values ranging from 29.4-102.3%, depending on the material considered. The addition of 4mM NAC was sufficient to lead to a full recovery of cell viability for some materials (SonicFill, X-traB), while the viability remained quite low for others (X-traF, SDR). Fill-up! could not be considered for this experiment due to its additional chemical cure.

DISCUSSION

To our knowledge, this is the first study performing a layer-by-layer evaluation of such a large array of commercially-available bulk-fill composites that combined a mechanical and biological characterization.

With regard to the first goal of this work, it appeared that the quality of cure in depth of commercially available composites is both material- and property-dependent. This can account for the inconsistency reported in the literature regarding the determination of the depth of cure of bulk-fill composites since all properties do not necessarily evolve similarly with depth.[26] Our results also underline the interest to combine various methods allowing the characterization of key mechanical and biological properties to provide a more representative picture of the performances of a material or group of materials with regards to their quality of cure in depth. For example, the conversion of bulk-fill composites has in the past been characterized mostly based on DC or microhardness measurements,[27-31] which does not reflect sufficiently their performance when laid in thick layers. This reinforces the need to combine complementary methods to characterize the depth of cure demonstrated in the past, notably to reduce the risk of overestimating the maximum material layer thickness.[19,32]

Not only is the maximum recommended thickness complicated to evaluate accurately by scientists, but also difficult to control accurately in practice during the layering process. Therefore, clinicians may use excessive composite thicknesses, which underlines the need to investigate beyond the recommended maximum thickness. In that sense, the lower (4-6 mm) and uncured layers were considered in the present work as, respectively, off-label use and a worst-case scenario.

As mentioned above, bulk-fill composites are usually considered as materials allowing polymerization in

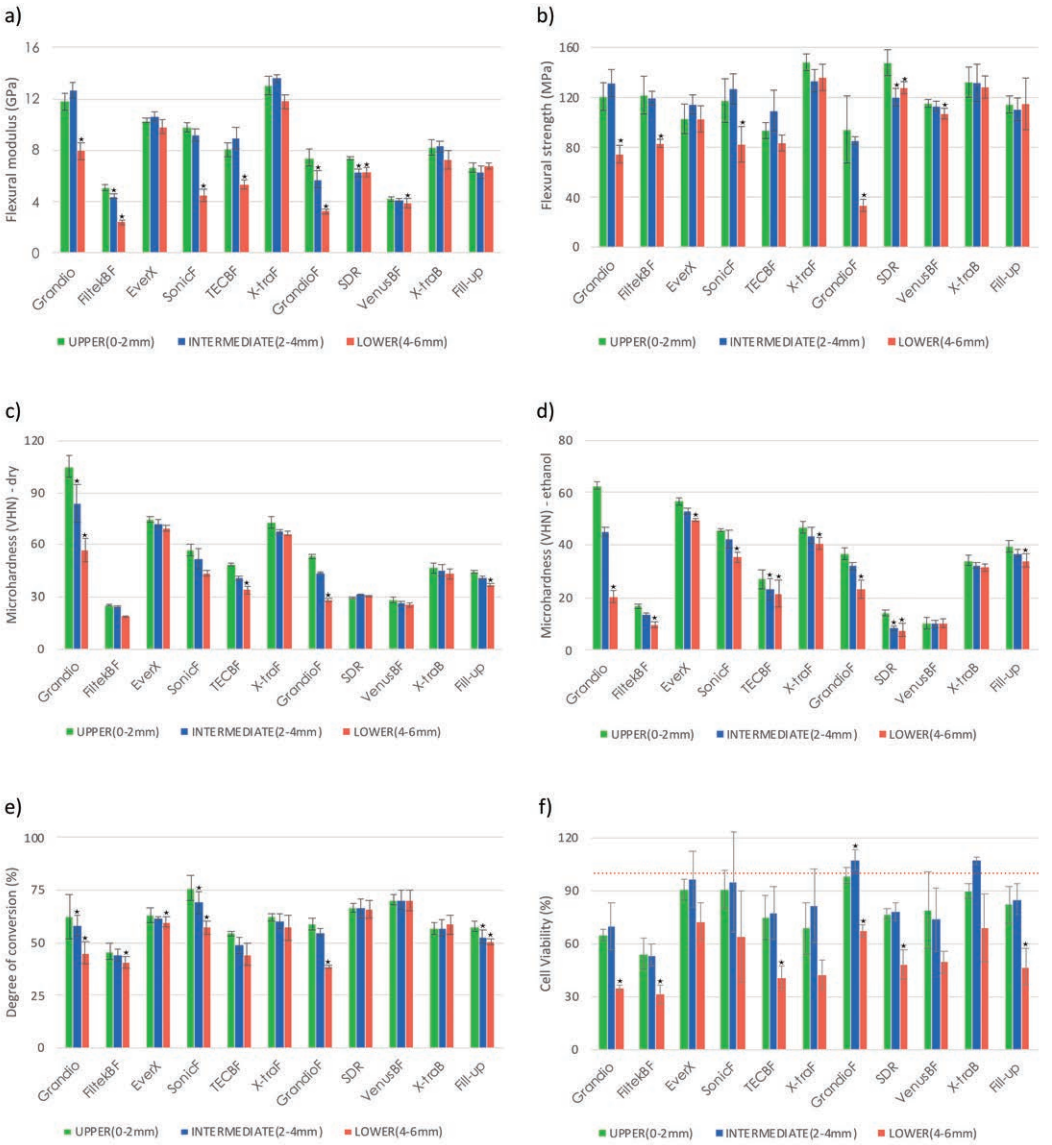

Figure 2. *Mechanical and biological properties for each material according to the three layers (upper, intermediate, and lower): (a) flexural modulus; (b) flexural strength; (c) microhardness after dry storage; (d) microhardness after 24 hours of ethanol storage; (e) degree of conversion; and (f) human dental pulp cell viability when grown in conditioned media, 100% viability (horizontal dotted line) corresponding to the values measured for the cells cultivated in regular growth medium; stars above the histograms indicate a significant difference (p<0.05) between the intermediate or lower layer and the upper layer considered as reference.*

4-mm thick layers. Within the presently considered materials, only four materials (EverX, X-traF, VenusBF, X-traB) presented no significant reduction between the upper and intermediate layers (≤ 4 mm) for all tested properties, which supports the recommendation made in previous work to be cautious with the bulk fill "label." [29] In fact, this labeling is further challenged by the fact that significantly increasing the curing time (100 seconds or more) may lead to comparable levels of conversion at 4-mm depth.[11]

It must be mentioned, however, that both in classic and recent work, the mold material was shown to have a significant impact on the depth of cure.[33,34] The choice of white Teflon in the present investigation was made to be closer to the remaining dental tissue in terms of light transport. However, a lower polymerization quality in depth would be expected when using metal molds, which better simulate the situation of a metal matrix band. Therefore, the quality of cure measured here in the depth of the material should not be extrapolated to all clinical situations, particularly not to those where a metal matrix is used. Also, it should be noted that the use of various types of molds represents a limitation preventing a direct correlation between the various

Table 2: *Inter-Material Properties Comparison for the Upper (0-2 mm) Layer (Means and Standard Deviations); Similar Letters within Columns Connect Materials Which are not Statistically Different* (p>0.05) *for the Considered Property*

Material	Flexural Modulus (GPa)			Flexural Strength (MPa)			DC (%)			Microhardness (VHN) Dry			Microhardness (VHN) EtOH			Cell Viability (%)		
	M	SD		M	SD		M	SD		M	SD		M	SD		M	SD	
Fill-up	6.7	0.4	D	114.3	6.9	CDE	57.9	2.3	DE	44.5	2.2	D	39.5	0.8	BC	82.3	10.4	AB
EverX	10.3	0.2	B	102.8	11.9	DE	63.1	1.3	CD	74.6	3.2	B	56.7	1.6	A	90.7	5.6	AB
FiltekBF	5.2	0.2	E	121.7	15.0	ABCD	45.7	0.7	F	25.2	3.9	E	16.9	0.2	E	54.5	8.5	C
Grandio	11.8	0.6	A	120.6	11.0	ABCDE	62.5	1.4	CD	105.1	10.7	A	62.6	6.3	A	65.0	3.6	BC
GrandioF	7.4	0.7	CD	94.2	26.9	DE	58.9	2.1	DE	53.1	2.9	CD	36.7	1.5	C	98.7	4.8	A
SDR	7.4	0.1	CD	147.8	10.4	A	66.9	1.0	BC	29.5	2.2	E	14.3	0.5	E	77.0	3.1	ABC
SonicF	9.8	0.4	B	117.4	17.5	BCDE	76.1	0.7	A	56.8	6.0	C	45.6	3.7	B	90.6	10.8	AB
TECBF	8.1	0.5	C	93.4	6.5	E	54.4	3.8	E	48.5	1.1	CD	27.0	0.7	D	74.8	12.3	ABC
VenusBF	4.3	0.2	E	115.1	3.2	CDE	70.4	2.1	AB	28.1	2.2	E	10.3	1.6	E	79.2	21.9	ABC
X-traB	8.2	0.6	C	132.2	12.0	ABC	57.0	2.1	DE	46.5	2.7	CD	34.1	3.1	CD	89.6	4.2	AB
X-traF	13.1	0.7	A	148.1	6.6	AB	62.1	2.3	D	72.8	1.5	B	47.0	3.6	B	68.7	14.5	BC

Abbreviations: M, mean; SD, standard deviation.

properties measured. Future work should consider designing experiments allowing the measurements of key physical, mechanical, and biological properties in depth on the same samples.

Not only does the evolution of a given property in depth need to be considered about the upper layer, but also the actual absolute value. To illustrate that, among the four materials strictly complying with "bulk-fill" criteria, very large differences could be observed, notably between X-traF and VenusBF. Hence, not only is the effect of depth to consider when choosing a material, but also and especially inter-materials differences.

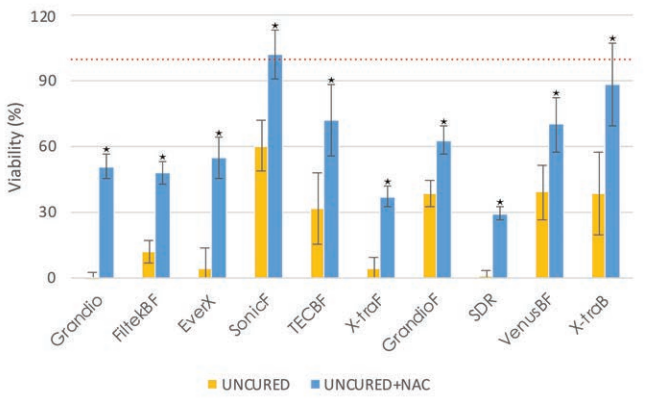

Figure 3. *Relative cell viability of cells grown in conditioned medium prepared with uncured composites, with and without the addition of 4 mM of NAC; 100% viability (horizontal dotted line) corresponds to the values measured for the cells cultivated in regular growth medium. *Identifies a statistical difference between uncured and uncured+NAC.*

This is in line with previous work based mostly on physico-mechanical aspects,[15,26] complemented here by the effect of composite conditioned media on hDPC viability, with similar conclusions.

It is well known that the conversion of dimethacrylate-based resins and composites is never complete,[35] and that substances can be released that have harmful effects on human pulp cells.[36] The cytotoxicity of the composite leachates depends both on the nature and the amount of the released molecules.[37] Monomers and compounds of the photoinitiator system represent most of the released molecules,[38] each molecule being associated with a different toxicity level. For example, the EC50 (concentration of substance necessary to kill 50% of a cell population) measured on human gingival fibroblasts varied from 0.087 mM for BisGMA to 11.53 mM for HEMA and 3.460 mM for TEGDMA.[37] Hence, a first possible explanation of the inter-material differences observed here regarding hDPC viability is logically the specific monomer composition of the composites. The ones containing the compounds with the lowest EC50 are most likely to lead to a more important drop in cell viability. Moreover, synergistic interactions between monomers are also possibly at play, since they were shown to potentiate cytotoxicity.[39] However, an additional parameter is essential regarding the cytotoxicity of a molecule, ie, its solubility in an aqueous medium. To induce cell damage, they must indeed be soluble in the aqueous growth medium. For example, the solubility of BisGMA in water is 9.5 ug/mm^3 as

compared to 27.5 ug/mm^3 for TEGDMA.[40] It was very elegantly demonstrated by Meng and others that no Bis-GMA could be detected in water storage after one week, while the monomer was detected in large amounts one week later once the solvent was switched to ethanol.[41] Consequently, the measurement of EC50 of hydrophobic monomers such as Bis-GMA required their prior dissolution into DMSO and subsequent dilution in an aqueous cell growth medium.[37] Since the growth medium without DMSO was used in the present work (aqueous media, 7 days extraction) to maximize clinical relevance, it is very likely that some hydrophobic compounds could not leach out of the composite due to their lack of hydrophilicity.

Last, although most of the cytotoxicity of resin-based composites has been attributed to monomers via oxidative stress,[23] the involvement of other components than the monomers cannot be ruled out. It has notably been shown that photoinitiators are released from cured composites, in various quantities depending on the extraction medium,[20] and that they can contribute to various extents to the dimethacrylate-based material cytotoxicity.[42,43] The presence of unconverted photoinitiator molecules was shown to result from insufficient light exposure,[44] which may be the case beyond 4-mm depth and explain the reduced cell viability of the lower layer.

It remains impossible to determine the relative part played by the aforementioned variables on cell viability due to the commercial nature of the materials. Despite being clinically relevant to investigate the materials used by practitioners, this remains a limitation of any work on commercial materials because their precise composition is rarely disclosed.[45] A full quantification of the monomers released would be useful to discuss certain hypotheses, but this is beyond the scope of the present work, and would in any case be much more accurate if the experiment were repeated with experimental materials of known composition.[8]

Finally, one needs to consider the layer effect (upper, intermediate, and lower) on hDPC viability. Both monomer-polymer conversion and cross-linking density of the polymer network were shown to affect the way unconverted monomers leach out of the material,[7] thereby affecting cell viability.[46] Hence, the first and most obvious explanation for the reduction in cell viability beyond 4-mm thickness would be the reduction in monomer conversion, which is known to be inversely correlated with monomer elution.[47] This is globally in line with the present data, and in agreement with previous work, including one bulk-fill and three conventional composites, and reporting no differences in viability until 4-mm depth.[48]

However, it can be noticed that the variations in DC between layers are generally smaller than those observed for hDPC viability. This can be related to the rather complex relationship existing between polymer conversion and cross-linking density. A rather large polymer network heterogeneity has been reported for di-methacrylate systems, which can include highly and loosely crosslinked domains.[35] Consequently, even at a similar DC, a different local cross-linking density can be measured.[19,32] This might affect both the proportion of the various co-monomers bound to the polymer network and their ability to diffuse out of the material.

Concerning the addition of 4 mM NAC, it was shown efficient to restrain the adverse effects of the released components on cell viability. NAC is an antioxidant, and the improvement of cell viability observed when it was added to the conditioned media is consistent with the observation of other articles.[49,50] Nevertheless, under the present experimental conditions, the addition of NAC at maximum non-cytotoxic concentration did not allow a full recovery of hDPC viability except for SonicF. This could first be explained by the fact that the maximum concentration of NAC remains too low (4 mM) to overcome all the generated reactive oxygen species. The second explanation is that monomers could exert their cytotoxic effect through another pathway. It has indeed been described that the increased levels of reactive oxygen species similar to those induced by 2-hydroxyethyl methacrylate were not associated with an increase in cell death or cell growth inhibition, unlike when the monomer was present.[51] This tends to indicate that monomer-induced cell damage may not be caused exclusively by the increase in reactive oxygen species.

CONCLUSIONS

Within the limitations of this work, and about the objectives, it can be concluded that:

1. There is an interest to combine various key mechanical and biological characterization methods to provide a more comprehensive picture of the performances of a group of materials. This concerns both their quality of cure in depth, which is material and property-specific, and their properties when "optimally" cured.
2. Not only does the evolution of a given property in depth need to be considered about the upper layer, but also the actual absolute value since significant inter-material differences were observed.
3. The use of NAC appears beneficial for reducing the risk of the harmful effect on dental pulp cells, especially in case of excessive thickness use, and

may be of potential interest as an additive to composites in the future.

Acknowledgments

The authors would like to thank the manufacturers for supplying us with the commercial materials investigated. JG Leprince thanks Cliniques universitaires St-Luc (Brussels, Belgium) for his FRC fellowship.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of UCLouvain, Brussels, Belgium. The approval code was #2013/16DEC/550.

Conflict of Interest

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

(Accepted 29 September 2021)

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Retention of Manually or CAD/CAM-customized Fiberglass Posts Luted to Enlarged Root Canals with Different Resin Cements

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Clinical Relevance

Customized fiberglass posts decrease resin cement thickness and void formation, favoring higher retention in enlarged root canals.

SUMMARY

The aim of this laboratory study was to evaluate the pull-out force of a prefabricated fiberglass post (PP), relined fiberglass post (RP), or milled fiberglass post (MP) luted with Multilink N (MN), RelyX Unicem 2 (RXU2) or RelyX Ultimate (RU) to enlarged root canals. The thickness of the resin cements and the presence of voids in the resin cement film were observed. The root canals of 90 bovine incisors were enlarged, endodontically treated, and randomly divided into 9 groups (n=10) according to the post type and resin cement. The specimens were scanned using micro-CT to analyze the thickness of the resin cement and the presence of voids. The specimens were submitted to mechanical cyclic loading (500,000 cycles at 50 N load) and subjected to pull-out force testing. Two-way ANOVA and Tukey's test analyzed the pull-out force and resin cement thickness data. Kruskal-Wallis and Bonferroni tests analyzed

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http://doi.org/10.2341/21-094-L

the void scores. The interaction between factors (post x resin cement) was significant (p=0.0001) for the pull-out force. Higher pull-out forces were obtained for RP and MP compared to PP. The post factor was significant (p=0.0001) for resin cement thickness, which was higher for PP (1054 μm), followed by MP (301 μm) and RP (194 μm). More void formation occurred for PP, being less for RP, differing significantly among the posts. Post customization (RP and MP) decreased resin cement thickness and void formation, favoring a higher pull-out force. Resin cements requiring an adhesive application (MN and RU) favored higher pull-out force than self-adhesive resin cement (RXU2).

INTRODUCTION

Successful functional and aesthetic rehabilitation of endodontically treated teeth depends on the amount of structure remaining.[1] Root canal access and instrumentation lead to substantial loss of root dentin and often require an intraradicular retainer and a full crown restoration.[2] The function of the post is to promote retention and support for coronal tooth restoration.[1,2]

According to the literature, posts are classified as prefabricated or customized. The most commonly used prefabricated post is the fiberglass post, which has an elastic modulus similar to dentin and resin cement and allows a uniform stress distribution and absorption of stress along the root, minimizing the risk of fractures.[3,4] Fiberglass posts also have good aesthetic and optical properties that make them suitable for use in highly aesthetic regions.[5]

Laboratory studies have shown that the closer the post is to the root canal walls, the smaller the resin cement film and the greater the bond strength between the root and the resin cement.[6,7] Overprepared or large root canals present a great thickness of resin cement when prefabricated fiberglass posts are used, which results in increased polymerization shrinkage stress in the resin cement layer, contributing to the formation of bubbles, cracks, and spaces along the post interface.[7,8] These discontinuities reduce the retention of the fiberglass post and lead to subsequent debonding, which is the main reason for post failure.[9,10]

In an attempt to improve fiberglass post adaptation in large root canals, a technique was proposed consisting of relining the prefabricated post with a composite resin.[11,12] This technique provides good adaptation to the canal walls, facilitating a thin and uniform resin cement layer and greater retention.[12,13] However, manual customization produces multiple interfaces between the prefabricated post and composite resin, increasing the chances of failure.[14]

Recent studies have suggested the use of computer-aided design/computer-assisted manufacturing (CAD/CAM) systems for the customization of posts produced directly or indirectly. Zirconia posts were the first CAD/CAM fabricated. Although they present excellent aesthetics, the high elastic modulus of zirconia posts causes stress in root dentin, resulting in catastrophic root fractures.[15] Spina and others[16] tested a hybrid ceramic material, a nanoceramic resin composite, and an experimental fiberglass-reinforced epoxy resin fabricated by CAD/CAM and showed that these materials have attractive optical properties and excellent adaptation in the root canal. More recently, some reports have indicated that customized post and cores milled from fiberglass blocks are practical and efficient clinical alternatives.[17]

Different resinous materials can be used for luting fiberglass posts, such as self-adhesive resin cements or resin cements requiring an adhesive system.[18] Post retention depends on the adhesion between the post and resin cement as well as between the resin cement and root dentin. There is little scientific evidence regarding the bond strength of a CAD/CAM fiberglass post to root dentin using different resin cements.[19-21]

Therefore, this study aimed to evaluate the pull-out force of a prefabricated fiberglass post (PP), relined fiberglass post (RP), or milled fiberglass post (MP) luted with Multilink N (MN), RelyX Unicem 2 (RXU2), or RelyX Ultimate (RU) to enlarged root canals. Additionally, the thickness of the resin cements and the presence of gaps and bubbles (voids) in the resin cement film were analyzed by microcomputed tomography (μCT). The hypotheses of the study were that 1) the post type and 2) resin cement influence the pull-out force to root dentin and that the fiberglass post type influences the 3) resin cement thickness and 4) void formation.

METHODS AND MATERIALS

Experimental Design

Ninety permanent bovine incisors with similar root sizes, lengths, and open apexes were selected. The teeth were cleaned and stored in distilled water at 4°C. The crowns were removed with a low-speed diamond disc under cooling below the cementoenamel junction. A small mark was made on the cervical buccal surface of the root with a spherical diamond bur in order to locate this face in the cyclic mechanical loading test. The length of the roots was standardized to

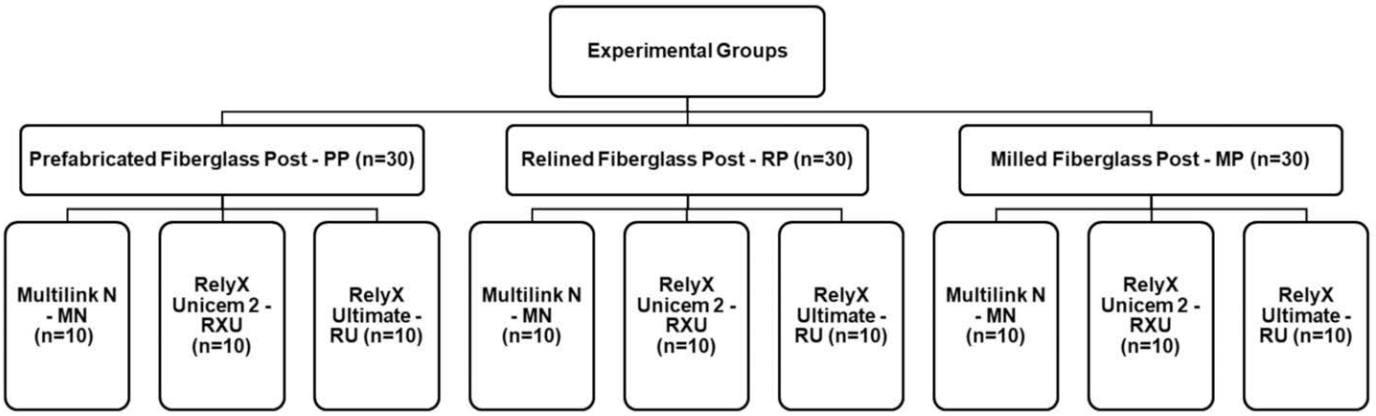

Figure 1. *Division of the groups in the study.*

16 mm. The canals were emptied, and the teeth were disinfected with 1% chloramine-T for seven days and then remained in distilled water.

The specimens were randomly divided into nine groups (n=10) according to the fiberglass post type—prefabricated fiberglass post #5 Exacto (PP), relined fiberglass post (RP), milled fiberglass post (MP), and resin cement—Multilink N (MN), RelyX Unicem 2 (RXU2), and RelyX Ultimate (RU), as shown in Figures 1 and 2. The materials used in this study are presented in Table 1.

Endodontic Treatment

A step-back preparation technique was performed for the endodontic treatment with stainless-steel #60 to #80 K-files and Gates Glidden #4 and #5 drills. All the enlargement procedures were followed by irrigation with a 2.5% sodium hypochlorite solution. A final irrigation with 17% EDTA was carried out for 5 min followed by washing with distilled water. The prepared root canals were filled with gutta-percha cones using lateral condensation and AH Plus resin sealer (Dentsply, Konstnaz, Germany). Then, the teeth were stored in 100% humidity at 37°C for seven days.

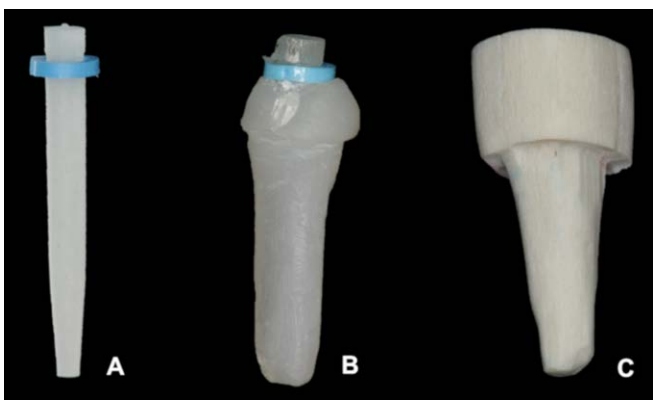

Figure 2. *Representation of post types: A - prefabricated fiberglass post (PP), B - relined fiberglass post (RP), and C - milled fiberglass post (MP).*

The gutta-percha was removed with a heated Rhein instrument (Golgran, São Caetano do Sul, SP, Brazil) until it reached 10 mm. The root canals were enlarged with a Largo #5 drill and high-speed diamond burs #4138 and #4137 (KG Sorensen, Cotia, SP, Brazil) with water irrigation. The remaining cervical dentin wall was approximately 1.0 mm thick, as measured with a digital caliper.

Post Preparation

Relined post (RP)—Exacto #5 posts (Angelus, Londrina PR, Brazil) were cleaned with 70% alcohol and gently air-dried. A layer of silane was applied to the surface of the posts for 1 minute and air-dried. A layer of Adper Single Bond 2 (3M ESPE, St Paul, MN, USA) was applied to the post surface, and the adhesive was air-dried. The tip of a Radii Cal curing light (SDI, Bayswater, Vic, Australia) was positioned in the apical portion of the post and the adhesive was light-cured for 10 seconds with a light intensity of 1000 mW/cm^2 as assessed by a radiometer (Model 100 Demetron, Saint Louis, MN, USA). A nanohybrid resin composite (A2D, Filtek Z350 XT, 3M ESPE, St. Paul, MN, USA) was used to customize the post. The composite resin was placed on the surface of the post and inserted into the root canal previously isolated with a water-soluble gel (KY Gel, Johnson & Johnson, São José dos Campos, SP, Brazil). The RP was light-cured for 20 seconds into the root canal. The RP was removed from the root canal, the tip of the light-curing unit was positioned in the apical portion of the post and the composite resin was light-cured for an additional 20 seconds. KY Gel was rinsed for 30 seconds after the relining procedure.

Milled post (MP)—The root canal was covered with CEREC Optispray (Cerec Optispray, Sirona, Bensheim,

Table 1: *Materials, Composition, Batch Number, and Manufacturer*[a]

Material/ Manufacturer	Composition	Batch
Exacto #5	Conical fiberglass post with 80% of glass fiber and 20% of epoxy resin	41799 100493
Fiber Cad	Glass fiber (75-80%) and epoxy resin (20-25%) block for CAD/CAM system	45769 45770
Multilink N	Primer A: 2,2'-[(4- methylphenyl)imino]bisethanol Primer B: HEMA, phosphoric acid acrylate Base: Ytterbium trifluoride, ethyoxylated bisphenol Adimethacrylate, Bis-GMA, 2-HEMA, 2-dimethylaminoethyl methacrylate Catalyst: Ytterbium trifluoride, ethyoxylated bisphenol-A dimethacrylate, urethane dimethacrylate, 2-HEMA,dibenzoyl peroxide	W11558 X36488 X29757
RelyX Unicem 2	Base paste: glass powder treated with silane, 2-propenoic acid, 2-methyl 1,10-(1- [hydroxymetil]-1,2- ethanodlyl) ester dimethacrylate, TEGDMA, silica-treated silane, glass fiber, sodium persulfate and per-3,5,5-trimethyl hexanoate t-butyl; Catalyst paste: glass powder treated with silane, substitute dimethacrylate, silica treated silane, sodium p-toluenesulfonate, 1- benzyl-5-phenyl-acid barium, calcium, 1,12- dodecane dimethacrylate, calcium hydroxide, and titanium dioxide	1811500433 1805800425
RelyX Ultimate	Base paste: Silane--treated glass powder, 2-propenoic acid, 2-methyl-, reaction products with 2-hydroxy-1,3-propanedyl dimethacrylate and phosphorus oxide, TEGDMA, silane--treated silica, oxide glass chemicals, sodium persulfate, tertbutyl peroxy-3,5,5- trimethylhexanoate, copper acetate monohydrate Catalyst paste: Silane-treated glass powder, substituted dimethacrylate, 1,12-dodecane dimethacrylate, silane--treated silica, 1-benzyl-5-phentyl-barbic-acid, calcium salt, sodium p-toluenesulfinate, 2-propenic acid, 2-methyl-, di-2,1-ethanediyl ester, calcium hydroxide, titanium dioxide	1808500058 3472648
Scotchbond Universal	BisGMA, HEMA, decamethylene dimethacrylate, ethanol, water, silane-treated silica, 2-propenoic acid, methacrylated phosphoric acid, copolymer of acrylic and itaconic acid, ethyl-4- dimethylaminobenzoat, camphorquinone, (dimethylamino) ethyl methacrylate, methyl ethyl ketone	3296401
RelyX Ceramic Primer	Ethanol, water, 3-methacryloxypropyltrimethoxysilane	N878550
Monobond-S	Ethanol, water, 3-methacryloxypropyltrimethoxysilane	X21804
Filtek Z350 XT	Bis-GMA, UDMA, TEGDMA, Bis-EMA, zirconia and silica nanoparticles (78.5 wt%/ 59.5 vol%)	896960
Adper Single Bond 2	BisGMA, HEMA, UDMA, dimethacrylates, ethanol, water, camphorquinone, photoinitiators, polyalkenoic acid copolymer, 5-nm silica particles	N688653

Abbreviations: HEMA, hydroxyethyl methacrylate; BisGMA, bisphenol A-glycidyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; Bis-EMA, ethoxylated bisphenol-A dimethacrylate; UDMA, urethane dimethacrylate.

[a]*The chemical composition information was obtained from the manufacturer's material safety data sheet.*

Germany) and scanned with CEREC Omnicam (Sirona, Bensheim, Germany). The root canal was rinsed with distilled water with the aid of a syringe for 30 seconds to remove the Optispray. The digital 3D model was created and designed with Cerec 4.6 software (Sirona, Bensheim, Germany). The fit of the post was planned at 100 μm. The block of Fiber CAD was milled in an inLab MC XL machine (Sirona, Bensheim, Germany).

Table 2: *Bonding Procedures Applied in the Experimental Groups*

Resin Cements/ Activation Mode	Dentin Pre-treatment	Post and Relined Post Treatment (PP, RP, MP)
Multilink N (MN) Self-cure	Multilink N Primers A and B were mixed at a 1:1 ratio and applied to the root canal with a microbrush, agitated for 30 s, and air-dried for 5 s. Excess material was removed with paper points.	A layer of Monobond-S was applied to the post surfaces with a microbush, left undisturbed for 60 s, and gently air-dried for 5 s.
RelyX Unicem 2 (RXU2) Dual-cure	No treatment, only a rinse with water, and excess water was removed from the root canal with absorbent paper points.	RelyX ceramic primer was applied to the post surface with a microbrush, left undisturbed for 60 s, and gently air-dried for 5 s.
RelyX Ultimate (RU) Dual-cure	Scotchbond Universal was applied into the root canal with a microbrush, agitated for 20 s, and gently air-dried for 5 s.	Scotchbond Universal was applied to the post surfaces with a microbrush, agitated for 20 s, and gently air-dried for 5 s.

Bonding Procedures

The bonding procedures applied in the experimental groups are described in Table 2. The mixing procedure of all the resin cements was standardized. Equal quantities of base and catalyst pastes of the resin cements were hand-mixed for 20 seconds and inserted into the canal using a Centrix syringe (NOVA DFL; Rio de Janeiro, RJ, Brazil) with AccuDose Tips. The RXU2 and RU resin cements were light-cured for 40 seconds according to the manufacturer's instructions, except MN, which was applied as a self-cured resin cement.

Micro-CT Images

After the bonding procedures, the samples were stored at 100% relative humidity at 37°C for 24 hours. The samples were scanned using a micro-CT (μCT) scanner (model 1173; Skyscan, Kontich, Belgium). The μCT was calibrated to operate under conditions of 85 kV and 65 μA, with an image pixel size of 9 μm, a rotational step of 0.22°, and an 800-ms exposure time. The average number of slices per specimen was 2150. For each specimen, 655 to 677 TIFF images were obtained. The obtained images were reconstructed using NRecon (Skyscan, Kontich, Belgium) software as demonstrated in Figure 3.

Resin Cement Thickness and Void Formation

The resin cement thickness was measured using Data Viewer software (Skyscan, Kontich, Belgium). The images were analyzed in a sagittal view (Z-Y). The coronal view (X-Z) and transaxial view (X-Y) were centralized at the center of the fiberglass post. Twenty equidistant measurements of the thickness of the resin cement film between the canal wall and the post were taken, as shown in Figure 4.

The images were also analyzed for the presence of voids and classified according to the extension and number of voids using the following scores: 0 = almost imperceptible voids; 1 = few and small voids in the resin cement film; 2 = many and small voids in the resin cement film; 3 = few and large voids in the resin cement film; and 4 = many and large voids in the resin cement film.

Cyclic Mechanical Loading and Pull-Out Testing

The roots were embedded with a self-cured acrylic resin (Jet Clássico, São Paulo, SP, Brazil) in a PVC cylinder that was 15 mm high and 25 mm in diameter. Regardless of the different shapes and sizes of the

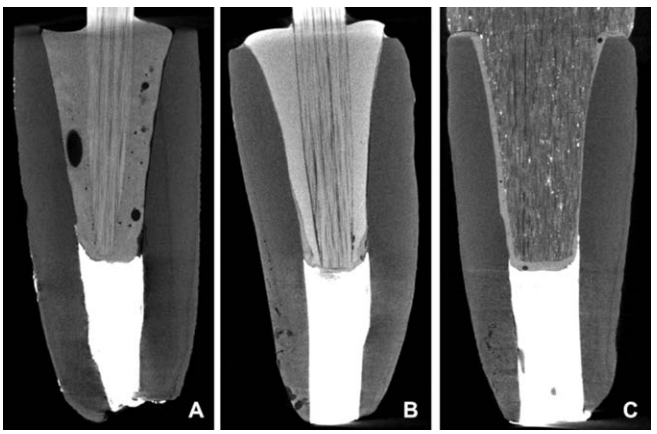

Figure 3. *Micro-CT images of fiberglass post types luted in the root canal: (A) prefabricated fiberglass post (PP); (B) relined fiberglass post (RP); and (C) milled fiberglass post (MP).*

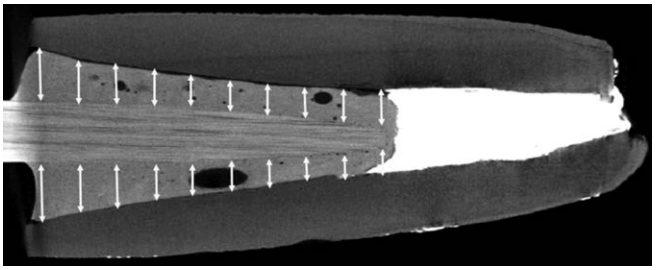

Figure 4. *Measurements of the resin cement thickness of a luted fiberglass post.*

coronal portions of the posts, it was necessary to apply composite resin on the facial face of the coronal portion of the post in order to serve as a niche for the plunger of the mechanical cycling machine. The samples were then submitted to cyclic mechanical loading (ER-11000, Erios, São Paulo, SP, Brazil) at 50 N using 500,000 cycles at 1 Hz in distilled water. The load was applied to the niche of composite resin at an angle of 45°.[22] After cycling mechanical loading, the samples were submitted to the pull-out force test.

The pull-out test was performed at a crosshead speed of 1.0 mm/min using a universal testing machine (EMIC, São José dos Campos, PR, Brazil) with a 1000-N load cell. The maximum load causing dislodgement of the post from the root canal wall was recorded in newtons (N). The mode of failure was assessed at 45× magnification under a stereomicroscope (SZH10, Olympus Corp., Tokyo, Japan) and was classified as follows: (a) adhesive failure at the dentin-resin cement interface; (b) adhesive failure at the post or relined post-resin cement interface; (c) mixed (combination of the failures "a" and "b" — resin cement covering parts of the post surface and parts of the dentin surface); (d) cohesive failure at the dentin; (e) cohesive failure at the post or relined post; and (f) adhesive failure at the post and relining composite resin interface.

Statistical Analysis

Two-way ANOVA (fiberglass post x resin cement) followed by Tukey's test was used to analyze the pull-out force data and resin cement thickness data. Pearson's correlation test was used to identify any correlation between the pull-out force and resin cement thickness. The void scores were analyzed by Kruskal-Wallis followed by Bonferroni tests. Statistical significance was set at 0.05. SPSS statistics 17 (IBM, ON, Canada) was used to carry out the statistical analyses.

RESULTS

According to two-way ANOVA, the fiberglass post factor ($p<0.001$), the resin cement factor ($p<0.001$), and the interaction between the factors ($p<0.001$) were significant for the pull-out force.

Comparing the posts for each resin cement, luting with MN provided a significantly higher pull-out force for RP (703±153 N). PP (344±142 N) and MP (404±136 N) did not differ significantly from each other. Luting with RXU2 promoted a significantly higher pull-out force for MP (510±124 N). PP (221±81 N) and RP (310±94 N) did not differ significantly from each other. For RU, the three types of posts differed significantly from each other, and RP (839±175 N) achieved the highest pull-out force, followed by MP (605±183 N) and PP (391±86 N) (Table 3).

Comparing the resin cements for each post, the pull-out forces of PP luted with RU (391±86 N) and MN (344±142 N) did not differ significantly and were significantly higher than that of RXU2 (221±81 N). The pull-out forces of RP luted with RU (839±175 N) and MN (703±153 N) did not differ significantly and were significantly higher than that of RXU2 (310±94 N). Higher pull-out forces were obtained for MP luted with RU (605±183 N) and RXU2 (510±124 N), which did not differ statistically from each other. The pull-out force of MP luted with RXU2 (510±124 N) did not differ significantly from MN (404±136 N) (Table 3).

Different modes of failure occurred in the groups (Table 4). Higher percentages of adhesive failure at the dentin-resin cement interface occurred for PP, RP, and MP luted with RXU2. For RP and MP luted with MN there was a predominance of adhesive failure at

Table 3: *Pull-out Force (N) and Standard Deviation of the Posts Luted to Root Dentin with the Resin Cements*[a]

	Prefabricated Fiberglass Post (PP)	Relined Fiberglass Post (RP)	Milled Fiberglass Post (MP)
Multilink N (MN)	344 ± 142 Ba	703 ± 153 Aa	404 ± 136 Bb
RelyX Unicem 2 (RXU2)	221 ± 81 Bb	310 ± 94 Bb	510 ± 124 Aab
RelyX Ultimate (RU)	391 ± 86 Ca	839 ± 175 Aa	605 ± 183 Ba

[a] *Mean values with different uppercase letters (rows) and different lowercase letters (columns) indicate significant differences according to Tukey's test (p<0.05).*

Table 4: *Mode of Failures (%) Observed for each Group*[a]

Mode of Failures	Adhesive at the Dentin-Resin Cement Interface	Adhesive at the Post or Relined Post-resin Cement Interface	Mixed	Cohesive at Dentin	Cohesive at Post or Relined Post	Adhesive at the Post and Relining Composite Resin Interface
MN + PP	1 (10%)	6 (60%)	3 (30%)	—	—	—
MN + RP	5 (50%)	3 (30%)	1 (10%)	1 (10%)	—	—
MN + MP	5 (50%)	—	5 (50%)	—	—	—
RXU2 + PP	7 (70%)	1 (10%)	2 (20%)	—	—	—
RXU2 + RP	10 (100%)	—	—	—	—	—
RXU2 + MP	10 (100%)	—	—	—	—	—
RU + PP	4 (40%)	2 (20%)	4 (40%)	—	—	—
RU+ RP	3 (30%)	4 (40%)	—	—	3 (30%)	—
RU + MP	—	2 (20%)	4 (40%)	4 (40%)	—	—

Abbreviations: MN, Multilink N; PP, prefabricated fiberglass post; RP, relined fiberglass post; MP, milled fiberglass post; RXU2, RelyX Unicem 2; RU, RelyX Ultimate

[a] *"— " indicates no failure.*

the dentin-resin cement interface. Most failures were adhesive at the post-resin cement interface for PP luted with MN. There was greater variability in the occurrence of the different failures for the samples luted with RU. Figure 5 shows the failures.

According to two-way ANOVA, the resin cement factor (p=0.481) and interaction between factors (p=0.743) were not significant for resin cement thickness. However, the fiberglass post factor (p<0.001) was significant. The three fiberglass posts showed significant differences. PP provided a higher resin cement thickness (1054±56 μm), followed by MP (301±19 μm) and RP (194±47 μm).

Pearson's correlation indicated an inverse relationship between the resin cement thickness and pull-out force (r^2=0.99; p<0.05) for all the resin cements (Figure 6).

According to the Kruskal-Wallis test, the resin cement factor was not significant (p=0.941) and the post factor was significant (p<0.001) for void formation. The Bonferroni test indicated that all the posts differed significantly from each other for extension and void quantity (Figure 7). Scores 2 and 3 were observed for PP, scores 1 and 2 for MP, and scores 0 and 1 for RP.

DISCUSSION

In the present study, all groups were considered experimental, without a control group, since various luting agents and corresponding adhesive systems have been proposed for bonding different types of fiberglass posts to root canal. The materials were selected because of the distinct etching methods and modes of polymerization.

The results showed that the pull-out force was significantly influenced by the post type and resin

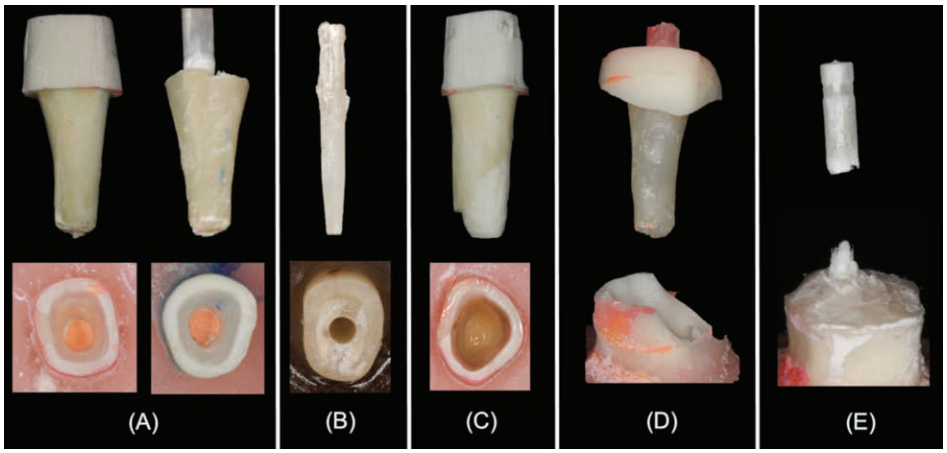

Figure 5. *Mode of failures: (A) adhesive at the dentin-resin cement interface; (B) adhesive at the post-resin cement interface; (C) combine or mixed; (D) cohesive at dentin; and (E) cohesive at post.*

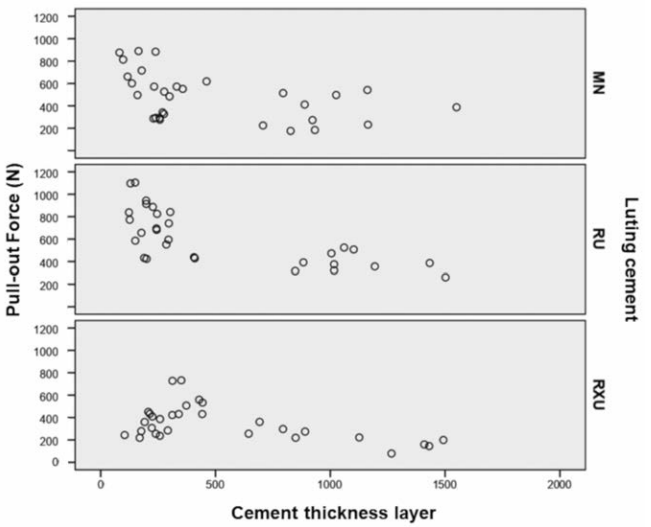

Figure 6. *Pearson's correlation between resin cement thickness and pull-out force.*

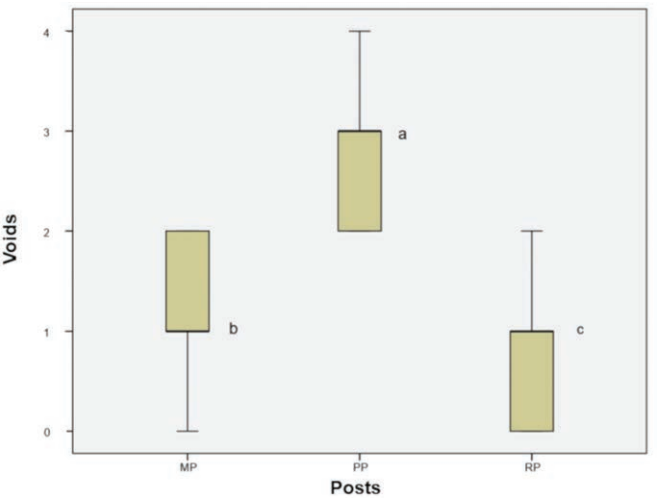

Figure 7. *Effect of fiberglass posts on the formation of voids in the resin cement. Different letters indicate differences according to Bonferroni's test (*p<*0.05).*

cement, leading to the acceptance of the first two hypotheses. PP luted with RU and MN showed a higher pull-out force than RXU2. RU requires the application of a universal adhesive, which was used in the self-etching technique in the present study. MN requires a self-etch primer prior to the application of resin cement. These approaches partially demineralize dentin, leaving a substantial amount of hydroxyapatite crystals around the collagen fibrils, providing mechanical retention through the formation of a hybrid layer and chemical bonding with specific carboxylic or phosphate groups of functional monomers.[23,24] In addition, the Scotchbond Universal adhesive system, previously applied to RU, contains the monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which allows a chemical bond to form between phosphate groups and residual hydroxyapatite crystals on the dentin collagen scaffold. This chemical bond reduces degradation of the hybrid layer over time and is more stable in water than the chemical bond obtained with other functional monomers.[23,25]

Beyond the pull-out force evaluation, it is also important to evaluate the mode of failures, as they represent the least resistant area to the stresses that occur during the pull-out test. Adhesive failures between PP and resin cement occurred in 20% of the RU specimens and 60% of the MN specimens. This mode of failure suggests that the pull-out force between the PP and resin cement was lower than that between the root dentin and adhesive material. In this study, the PP surface treatment followed the manufacturer's instructions. For luting with MN, the PP was treated with silane (Monobond S); for luting with RU, Scotchbond Universal adhesive system was applied on PP. It is possible that these failures would have been diminished if the post had been etched with hydrogen peroxide or sandblasted with aluminum oxide particles. These procedures enhance the surface roughness of the fiberglass post, exposing more glass fibers and increasing the bond strength to the adhesive agent.[26,27] There were also 40% mixed failures for RU and 30% for MN. This failure is characterized by the cohesive fracture of the resin cement itself, showing that the bond of the resin cement to the root dentin has overcome the cohesive strength of the material. Therefore, it is estimated that the pull-out force of RU and MN to the root dentin, which corresponds to adhesive failure between the substrate and resinous material (40% for RU and 10% for MN), could be higher if mixed or adhesive failure between the PP and resin cement had not occurred.

The luting of PP with RXU2 provided a lower pull-out force. RXU2 is a self-adhesive resin cement that presents methacrylate monomers containing phosphoric acid groups, which provide dentin conditioning. These monomers simultaneously demineralize hydroxyapatite and infiltrate the superficial dentin, providing micromechanical retention and chemical bonding that occurs from an acid-base reaction between the acid monomers and the dental substrate or acid-soluble inorganic fillers.[18,28,29] The low pull-out force obtained with RXU2 could be attributed to the low diffusion of the resin cement in the demineralized dentin and the absence of a hybrid layer or resin tags.[18,30] Although the PP surface was treated with silane alone for luting with RXU2, there was only 10% adhesive failure between the PP and resin cement, and most of the failures were adhesive between the dentin and resinous material

(70%). This result corroborates the lower pull-out force obtained with RXU2.

PP and RP luted with RU and MN obtained a greater pull-out force than RXU2. However, the pull-out force of RP was significantly higher than that of PP, and the Pearson's correlation test indicated an inverse relationship between the resin cement thickness and pull-out force. This finding is related to the significantly thinner resin cement layer obtained for RP than PP. Thus, the third hypothesis was accepted. This result was expected since PP does not fit the weakened root and the space between the post and the root walls is filled with resin cement. In contrast, RP is modeled with composite resin inside the root canal, allowing better adaptation to the root canal walls. The thin space between the root dentin and RP is filled with resin cement. Previous studies have demonstrated that the proximity between the root canal walls and RP increases the sliding friction[31] and improves post retention,[11] promoting a higher pull-out force.[8,13,32] In addition, RP pushes the resin cement against the root canal walls during the luting procedure, favoring tag formation into the dentinal tubules. These resin tags could favor an increase in pull-out force.[12] The poor adaptation between PP and the dentin root walls results in a thick resin cement layer that may lead to higher polymerization shrinkage and air entrapment, weakening the bond and increasing the possibility of post debonding.[7,8,33]

In RP, adhesive failure between the relined post and resin cement occurred in 40% of the RU specimens and 30% of the MN specimens. This failure took place between the composite resin used for the relining and the resin cement. This finding also demonstrates that a higher pull-out force could be obtained if another type of surface treatment of the composite resin covering the post had been carried out, such as air abrasion with alumina particles.[34] In the present study, the surface treatment of the relining composite resin followed the procedures used for PP and MP with the aim of standardization for each resin cement. However, there were 100% adhesive failures between the dentin and resin cement for RXU2, providing a lower pull-out force. The present study does not corroborate the result of other studies in which the use of this self-adhesive resin cement promoted a higher bond strength to the root dentin compared with resin cements requiring an adhesive.[22,35] One possible explanation for the different results is the methodology applied in the studies since most of them evaluated the bond strength through the push-out bond test and not the pull-out force test.[36]

Regarding the failures obtained in the RP group, there was no adhesive failure at the relining composite resin and fiberglass post. This result shows an adequate bond between the relining composite resin and fiberglass post, which was obtained by the application of silane and adhesive. An important step involving the RP technique is the KY gel application in the root canal to avoid the bond between the relining composite resin and root dentin. As this gel is water-soluble and was abundantly rinsed after the relining procedure, it is not expected it had any influence on the bonding of RP in the root canal.

MP presented an intermediate resin cement thickness (301 μm) compared with PP (1054 μm) and RP (194 μm). The milling spacer was set to 100 μm, which is very close to the cast post and core found in the study of Tsintsadze and others.[20] However, when analyzing the luting film along the root canal by microCT images, it was observed that it varies. These findings agree with the study of Prudente and others,[37] which showed a resin cement film ranging from 16 to 230 μm with a milling space of 70 μm. This finding can be explained by software compensation because of anatomical details, imperfections related to the preparation, and difficulties in the capture and construction of the image, increasing the misfit of the post.[38]

MP luted with RU showed a significantly greater pull-out force, not differing significantly from RXU2. Thus, the lowest pull-out force was obtained with MN, which did not differ significantly from RXU2. Although luting with RXU2 promoted an intermediate pull-out force for MP, 100% adhesive failures occurred between the resin cement and root dentin. This finding demonstrates the same tendency for adhesive failure that occurred for the PP and RP luted with RXU2. Thus, the pull-out force of this resin cement to root dentin did not exceed the bond strength between the post and the resin cement and did not overcome the cohesive strength of the resin cement itself. MP luted with RU resulted in 20% adhesive failures between the post and the resin cement. MP was only treated with the Scotchbond Universal adhesive for luting with RU. It is possible that an additional chemical or micromechanical surface treatment could increase the bond strength between the adhesive and the post, preventing this type of failure.[21] There were also 40% mixed failures and 40% dentin failures, showing strong bonding of the adhesive material to the root dentin. It is estimated that the pull-out force obtained for MP luted with RU exceeded the cohesive strength of dentin itself. For MN, 50% of adhesive failures occurred between dentin and the resin cement, and 50% were mixed failures. Therefore, it is also estimated that in 50% of the specimens, the pull-out force exceeded the cohesive strength of the resin cement itself. Thus, the resin

cements requiring adhesive demonstrated a greater capacity to promote retention of the posts inside the root canal.

The insertion of resin cement into the root canal and void formation influences the bond quality between the dentin walls and posts.[39,40] Void formation is mainly a result of air entrapment in the resin cement during the mixing process. Automatically mixed resin cement insertion reduces void formation in the material because the base paste and catalyst paste are not in contact with air during mixing. This reduction in void formation favors an increase in the bond strength of the resin cement to the substrate.[39-41] However, in this study, for standardization purposes, all the resin cements were hand-mixed and inserted into the root canal using a Centrix syringe. Silva and others showed that a Centrix syringe reduced apical void formation in comparison with hand-mixed resin cements and insertion using endodontic files. Additionally, the Centrix syringe allows a homogeneous resin cement interface.[39] Micro-CT analysis showed a certain number of voids inside all the tested groups, which was influenced by the fiberglass post type. Thus, the fourth hypothesis was accepted. PP presented many small voids and few large voids in the resin cement film. For RP, the voids were almost imperceptible, or there were a few small voids. For MP, there were either few or many small voids. These findings agree with previous studies.[8,41,42] These results may indicate that the post customization, obtained by RP and MP, minimizes the resin cement thickness layer[12,42] and void formation,[42,43] increasing the bond strength and enhancing the survival time of the post.[41]

Post retention has been evaluated by push-out and pull-out tests.[36] Post retention depends not only on chemical bonds provided by the luting agent but also on micromechanical interlocking and sliding friction.[31] Since one of the most common failures involving fiberglass posts is debonding,[9,10] the pull-out test was chosen for this study. The pull-out test allows a simultaneous evaluation of the shear and tensile stresses that develop during the test,[44-47] resulting in the force necessary for debonding the post surface in the entire length of the root canal, not only in root segments, such as in the push-out test. These characteristics of the pull-out test allow greater clinical relevance of the results.

It is important to emphasize that the pull-out force values may also have been influenced by the different modes of polymerization. Dual resin cements, such as RU and RXU2, are light-cured and chemically cured. These materials have been recommended to lute fiberglass posts, because the curing light is not able to ensure adequate polymerization in deep areas of the root canal.[48] However, the self-cured reaction of dual resin cements is not capable of totally compensating for poor polymerization in deep areas where light intensity is low, favoring a lower degree of conversion[49] and lower bond strength value in the apical third.[18,49] In contrast, self-cured resin cements, such as MN, are activated by the peroxide-amine system without requiring light exposure; hence, the polymerization is not influenced by root canal depth.[50]

Cyclic mechanical loading is a laboratory aging methodology that aims to submit the specimens to a cyclic load to reproduce the masticatory loads that are applied to the teeth. In the present study, the samples were submitted to 500,000 cycles with a 50 N load. In this way, approximately two years of normal functionality was simulated.[51,52] The load was applied to the post head that varied in shape among the posts, being larger for MP. It is possible that the force transmitted down the canal was different among the posts. Therefore, it cannot be ruled out that the different post heads have influenced the results of the pull-out force.

The results of the present study demonstrated that both RP and MP presented better performance in enlarged roots than PP. However, it is important to highlight that RP has numerous adhesive interfaces, increasing the chance of material degradation at the interfaces and, consequently, bond failures.[14,53] In contrast, MP is manufactured as a single piece without interfaces. A homogeneous fiberglass block is submitted to a controlled milling process and with a fully digital workflow.[16,19,42] Due to the lack of literature on MP, further studies should be conducted to evaluate the pull-out force of these posts to the root dentin under long-term conditions. In addition, studies related to the pretreatment of MP should be carried out.

CONCLUSIONS

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

1. Post customization, such as relined or milled fiberglass posts, decreased resin cement thickness and void formation and favored a higher pull-out force to enlarged roots compared to prefabricated fiberglass posts.
2. Resin cements requiring adhesive application (Multilink N and RelyX Ultimate) favored a higher pull-out force than self-adhesive resin cement (RelyX Unicem 2).

Acknowledgment

The authors are grateful to Coordination for the Improvement of Higher Education Personnel (*CAPES, Brazil*) for the scholarship for MCB (Finance Code 001).

Conflict of Interest

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

(Accepted 6 October 2021)

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Cement Choice and the Fatigue Performance of Monolithic Zirconia Restorations

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Clinical Relevance

The clinical fatigue performance of cemented monolithic zirconia can be influenced by the cement choice. Proper selection of the cement system can enhance long-term fatigue performance.

SUMMARY

This study investigated the fatigue failure load of simplified monolithic yttria partially stabilized zirconia polycrystal restorations cemented to a dentin-like substrate using different luting systems. Disc-shaped ceramic (Zenostar T, 10 mm Ø × 0.7 mm thick) and dentin-like substrate (10 mm Ø × 2.8 mm thick) were produced and randomly allocated into eight groups, without or with thermocycling (TC=5-55°C/12,000×): "cement" (RelyX Luting 2 – glass ionomer cement [Ion], [Ion/TC]; RelyX U200 – self-adhesive resin cement [Self], [Self/TC]; Single Bond Universal+RelyX Ultimate – MDP-containing adhesive + resin cement [MDP-AD + RC], [MDP-AD + RC/ TC]; ED Primer II+Panavia F 2.0 – Primer + MDP-containing resin cement [PR + MDP-RC], [PR + MDP-RC/TC])). Each luting system was used as recommended by the manufacturer. Staircase methodology (20 Hz; 250,000 cycles) was applied for obtaining the fatigue failure loads. Fractographic characteristics were

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http://doi.org/10.234/20-143-L

also assessed. At baseline, the Ion group presented the lowest fatigue load, although it was statistically similar to the Self group. The resin-based cement systems presented the highest fatigue performance, with the Ion group being only statistically equal to the Self group. Thermocycling influenced the groups differently. After aging, the MDP-AD + RC presented the highest mean, followed by the PR + MDP-RC and Self groups, while the Ion group had the lowest mean. Fractographic analysis depicted all failures as radial cracks starting at the zirconia intaglio surface. The luting system with MDP-containing adhesive applied prior to the resin cement presented the highest fatigue failure load after aging, presenting the best predictability of stable performance. Despite this, monolithic zirconia presents high load-bearing capability regardless of the luting agent.

INTRODUCTION

Monolithic zirconia restorations are an alternative to bilayer restorations in posterior teeth since they have not shown chipping or fractures after at least 68 months of clinical use,[1,2] and they allow less tooth reduction,[3,4] being used in reduced thickness (0.5-1.0 mm). Long-term clinical data on monolithic zirconia treatments are still scarce[5]; however, clinical studies with zirconia-based restorations have shown that fracture and retention loss are their main reasons for failure.[6-11]

When considering the factor "retention loss" for all-ceramic restorations, one of the aggravating characteristics is their internal relief. Unlike metal-ceramics, which have a certain primary friction to the dental substrate when cemented, all-ceramic crowns are not able to withstand such tension without damage.[12] According to Kelly,[12] this friction could induce tensile stress capable of generating internal cracks in the restoration, in addition to generating the radial tension effect (*Hoop stress*) caused by dental crowns' cylindrical shape when submitted to load (eg, luting procedure and chewing cycles). In this sense, the luting material plays an important role to compensate for this lack of primary friction and to prevent the restoration from debonding. Therefore, the choice of luting material should not be based on clinician preferences, but rather on scientific evidence that considers and compares specific protocols.

Several studies have demonstrated the benefits and importance of using techniques that not only promote a micromechanical bond, but also a strong, reliable and long-lasting chemical bond between tooth and ceramic restoration for greater longevity.[13-15] The quality of bonding interfaces is one of the major factors responsible for the fracture resistance of all-ceramic dental crowns since bulk fractures originate from defects on the restoration intaglio surface.[16] Also, these findings have been confirmed through fractographic analyses of clinically failed restorations[16] and using finite element predictions.[17] Therefore, it becomes clear that the use of different cement systems can potentially influence the retention and fatigue behavior of such restorations; factors which are largely related to the restoration longevity.[18]

The adhesive cementation should be performed whenever possible, as it generates higher bond and fatigue strength than conventional luting, which is related to a higher failure rate by retention loss.[11,19,20] Resin cements have better mechanical and optical properties, and higher resistance to abrasion and to hydrolysis,[21] despite their higher technical sensitivity and lower moisture tolerance. The introduction of functional monomers has improved the resin cement bond strength to zirconia,[13] but the effect of using different methacryloyloxy-decyl-dihydrogen-phosphate (MDP)-containing cement systems in the fatigue behavior of cemented monolithic zirconia is still unclear.

Moreover, the ability of luting systems to adequately fill the defects of the ceramic intaglio surface is another concern when cementing these restorations.[22] The fracture strength of ceramic materials is related to the size and number of defects present in their surface,[23] and unfilled defects can work as starting points for slow crack growth under constant masticatory stresses, and consequently cause early failure in the restoration.[24]

The hostile oral environment when associated to cyclic masticatory loads results in restoration failure due to long-term damage propagation, which constitutes fatigue failure.[25] This failure can be defined as ceramic fracture due to subcritical slow crack growth (SCG), which occurs under cyclic tensions lower than the normal strength of the material,[26] and SCG is accelerated in aqueous environments.[24] According to Ritter,[27] SCG can be explained as a chemical reaction that occurs between water and ceramics, by breaking their metal oxide bonds. In this way, the crack increases slowly, leading to strength decrease, and failure of restorations over time. Also, the cement's properties (eg, elastic modulus) may be affected by aging.[28]

From these standpoints, the aim of this study was to investigate the effect of using different luting systems on the fatigue performance of simplified monolithic zirconia specimens cemented to a dentin-like substrate, after applying stress by fatigue loading and thermal cycling. The null hypotheses tested were: 1)

cement type and 2) aging will not affect the zirconia fatigue performance.

METHODS AND MATERIALS

To eliminate some production expenses (ie, CAD/CAM milling) and the complications associated with creating predictable contact between the piston and anatomically contoured ceramic while allowing the evaluation of the factors in this study, a simplified assembly (disc-shaped samples) for producing the restorative specimens was used. That approach is well validated in the literature[29-32] since it produces a stress distribution very similar to clinical scenarios where the stress concentration is higher at the luting interface on the intaglio ceramic surface, triggering the origin of failure.

Study Design

A second-generation yttria-stabilized tetragonal zirconia polycrystal (Y-TZP; 4.5% to 6.0% yttria content; Zenostar T; Wieland Dental, Ivoclar Vivadent; Schaan, Liechtenstein) indicated for framework and monolithic prosthetic restorations was used in the present study. The zirconia thickness used in the study was the minimal recommended by the manufacturer for monolithic posterior crowns, being 0.7 mm. The zirconia discs were cemented on flat dentin-like substrate discs (woven glass-fiber-filled epoxy resin; National Electrical Manufacturers Association [NEMA] grade G10, Accurate Plastics Inc, New York, USA; E_{G10}= 18.6 GPa - elastic modulus similar to wet dentin E_{dentin}= 18 GPa[29]). The final diameter of the specimens was 10 mm resembling the mean diameter of the occlusal surface of the first permanent molar.[33] The final thickness of the whole specimen set was 3.5 mm (G10 discs=2.8 mm, zirconia discs=0.7 mm), being equivalent to the mean thickness between the occlusal surface and the dental pulp chamber roof.[34,35]

Production of Specimens

Y-TZP Ceramic Discs—Zenostar T discs (98.5 mm Ø × 16 mm in thick) (n=200) were manually cut into small blocks (12 × 12 × 16 mm^3) with a diamond disc coupled to a handpiece attached to an electric motor (Perfecta LA 623T, 1000 at 40,000 rpm; W&H, Bürmoos, Austria). Next, metallic rings (Ø=12 mm) were glued to the parallel surfaces of the small blocks to guide the grinding in a polishing machine (EcoMet/AutoMet 250, Buehler, Lake Bluff, IL, USA) with #600 grit silicon carbide papers (SiC) and water-cooling to obtain zirconia cylinders with 12 mm of diameter.

Then, 0.94-mm thick slices were obtained by cutting under water-cooling with a diamond blade (Buehler-Series 15LC Diamond; Buehler) in a precision cutting machine (Isomet 1000, Buehler), resulting in 200 discs. The discs were manually polished on both sides with SiC papers (#600 and #1200 grit) to obtain a smooth surface, free from defects and with a final thickness of 0.86 mm. They were subsequently cleaned (ultrasonic bath with distilled water for 10 minutes) and dried, and then sintered in a specific furnace (heating rate of 600°C/h; temperature 1 of 900°C with a holding time of 0.5 h; heating rate of 200°C/h; and temperature 2 of 1450°C with a holding time of 2h; VITA Zyrcomat 6000 MS, Vita Zahnfabrik, Bad Säckingen, Germany), followed by ultrasonic cleaning in 78% isopropyl alcohol for 5 minutes. The final dimensions of the zirconia discs were 10.0 mm in diameter and 0.7 (±0.02) mm in thickness.

Dentin-like Substrate Discs—NEMA G10 round rods (±250 mm length × 12.7 mm Ø) had their diameters reduced to 10 mm and then sliced in 3.0-mm thick discs (n=200) by the methodology previously described for the zirconia discs. After cutting, the discs were polished with SiC papers (#400 and #600 grit) until a final thickness of 2.8 mm, followed by ultrasonic cleaning in 78% isopropyl alcohol for 5 minutes.

Luting Procedure— Zirconia/Dentin-like Substrate

The intaglio surface of the all-zirconia discs was air-abraded for 10 seconds with aluminum oxide particles (Al_2O_3; 45 μm particle size) with oscillatory movements and a perpendicular angulation (90°) between the device tip and the specimen surface at a distance of 10 mm and at 2.8 bars of pressure. Next, the specimens were ultrasonically cleaned in distilled water for 5 minutes.

All the dentin-like substrate discs were etched with 10% hydrofluoric acid for 1 minute (HF etching), rinsed for 30 seconds, ultrasonically cleaned in distilled water for 5 minutes, and air-dried.

The specimens (zirconia and dentin-like substrate discs) were then randomly (www.randomizer.org) allocated into 8 groups (n=25) according to the study factors (cement and aging) (Table 1). The primers for each cement system were applied to the disc surfaces, and the cements were handled and applied following the manufacturers' instructions, as explained in Table 2.

After the primers were applied, each cement was mixed according to manufacturers' instructions (1:1 ratio) and applied on the dentin-like substrate disc. The zirconia discs were seated in their respective pairs under a uniform load of 250 g, which would be enough to produce a thin and uniform cement layer;[36] the cement excess was removed, and the cement was

Table 1: *Study Experimental Design*[a]

Cement Systems			Groups	
Classification	**Commercial Name (Brand), Elastic Modulus**	**General Composition**[b]	**Baseline**	**Aged (TC)**
Resin-modified glass ionomer cement (1-step)	RelyX Luting 2 (3M Oral Care) *E*=4 GPa[b]	Cement - Paste A: radiopaque FAS glass, proprietary reducing agent for self-cure, HEMA, water, opacifying agent; Paste B: methacrylated polycarboxylic acid, HEMA, water, potassium persulfate, non-reactive zirconia silica filler.	Ion	Ion/TC
Self-adhesive resin cement (1-step)	RelyX™ U200 (3M Oral Care) *E*=6.6 GPa[b]	Cement - Base paste: methacrylate monomers containing phosphoric acid groups, methacrylate monomers, silanated fillers, initiator components, stabilizers, rheological additives. Catalyst paste: methacrylate monomers, alkaline (basic) fillers, silanated fillers, initiator components, stabilizers, pigments, rheological additives	Self	Self/TC
Self-etching primers + MDP-containing adhesive resin cement (2-steps)	ED Primer II + Panavia F 2.0 (Kuraray Noritake) *E*=18.3 GPa[c]	Cement - Paste A: 10-MDP, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated silica filler, silanated colloidal silica dl-Camphorquinone, catalysts; Paste B: hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated barium glass filler, catalysts, accelerators, pigments. Primers - Liquid A: HEMA, 10-MDP, N-methacryloyl-5-aminosalicylic acid, water, accelerators. Liquid B: N-methacryloyl-5-aminosalicylic acid, water, catalysts, accelerators	PR + MDP-RC	PR + MDP-RC/TC
MDP-containing universal adhesive + adhesive resin cement (2-steps)	Single Bond Universal + 3M RelyX Ultimate (3M Oral Care) *E*=7.7 GPa[b]	Cement - Base paste: methacrylate monomers, radiopaque, silanated fillers, initiator components, stabilizers, rheological additives. Catalyst paste: methacrylate monomers, radiopaque alkaline (basic) fillers, initiator components, stabilizers, pigments, rheological additives, fluorescence dye, dark cure activator for Scotchbond Universal Adhesive (3M Oral Care). Adhesive - MDP, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	MDP-AD + RC	MDP-AD + RC/TC

Abbreviations: FAS, fluoroaluminosilicate; HEMA, 2-hydroxyethylmethacrylate; MDP, methacryloyloxydecyl-dihydrogen-phosphate; TC, thermocycling; AD, adhesive; RC, resin cement.

[a]*Cement Systems: classification, commercial name, brand and elastic modulus, and general composition; aging: baseline and aged (Thermocycling - TC: 12,000 cycles between 5 ºC and 55 ºC, 30 seconds dwell time; 4 seconds transfer time); and group codes.*

[b]*Manufacturer's data.*

[c]*Li and others*[60]

Table 2: *Luting Procedures for the Different Cement Systems*

Cement	Groups	Surface Treatment	
		Epoxy Resin	**Zirconia**
Resin-modified glass ionomer cement (1-step); RelyX Luting 2	Ion and Ion/TC	After HF etching (see the luting procedure section), a silane-coupling agent[29] (RelyX Ceramic Primer; 3M Oral Care) was applied for 5 s, and gently air-dried.	After air-abrasion and cleaning (see the luting procedure section), the specimens were vigorously air-dried.
Self-adhesive resin cement (1-step); RelyX U200	Self and Self/TC		
Self-etching primers + MDP-containing adhesive resin cement (2-steps); ED Primer II + Panavia F 2.0	PR + MDP-RC and PR + MDP-RC/TC	After HF etching (see the luting procedure section), the Panavia system ED Primers II, liquids A and B, were mixed (ratio 1:1) and applied on the surface, the mixture was left to react for 30 s and primer excess was removed by gentle air-drying for 5 s.	After air-abrasion and cleaning (see the luting procedure section), the specimens were vigorously air-dried.
MDP-containing universal adhesive + adhesive resin cement (2-steps); Single Bond Universal + RelyX Ultimate	MDP-AD + RC and MDP-AD + RC/TC	After HF etching (see the luting procedure section), the Single Bond Universal Adhesive (3M Oral Care) was applied and left to react for 20 s and the excess was removed by gentle air-drying for 5 s.	After air-abrasion and cleaning (see the luting procedure section), the specimens were vigorously air-dried. Single Bond Universal Adhesive (3M Oral Care) was applied and left to react for 20 s and the excess was removed by gentle air-drying for 5 s.

Abbreviations: Ion, glass ionomer cement; Self, self-adhesive resin cement; MDP-AD + RC, MDP-containing adhesive + resin cement; PR + MDP-RC, Primer + MDP-containing resin cement; TC, thermocycling; MDP, methacryloyloxydecyl-dihydrogen-phosphate.

light-cured (1200 mW/cm^2, 440-480 nm, Radii-cal, SDI; Bayswater, Australia) for 20 seconds through the occlusal ceramic surface and for 20 seconds on each side (0º, 90º, 180º and 270º) of the specimen set.

Artificial aging - Thermocycling 'TC'

Half of the specimens from each cement system underwent 12,000 thermal cycles between two water baths, 5°C and 55°C (30 seconds dwell time and 4 seconds transfer time (model 521-6D, Ethik Technology, Vargem Grande Paulista, Brazil), 1 day after cementation and stored for four days after thermocycling. After cementation, the specimens not thermocycled were stored in distilled water at 37ºC in a laboratory oven (Laboratory Thermo incubator, Model 502, FANEM, São Paulo, Brazil) for four days.

Fatigue Failure Load Testing - Staircase Method

The specimens for each group were numbered and randomized (www.randomizer.org) to determine their test sequence. The fatigue tests were executed in an electric machine (Instron ElectroPuls E3000, Instron Corp, Norwood, MA, USA) over a flat steel base and through the Staircase sensitivity method.[37] The cyclic loads (250,000 pulse cycles; 20 Hz frequency; wet testing) were applied to the center of the disc surface on the zirconia side by a 40-mm Ø hemispheric stainless-steel piston (Figure 1).[29,38] The fatigue test parameters (initial load = ~60% of the mean of load-to-failure tests; and step-size = ~5% of the initial load) were obtained from the mean of the static load-to-failure tests (0.5 mm/min crosshead speed [EMIC DL 2000, São José dos Pinhais, Brazil] of 5 specimens until the specimen's failure, ie, auditory perception of cracking by a single trained operator). This procedure was performed for each group. An adhesive tape (110 μm thick) was placed on the zirconia surface to improve stress spreading during load application,[39,40] and a polyethylene sheet (10 μm thick) was placed between the piston and the cemented set to reduce contact stress concentration,[41] both in order to avoid contact damage (Hertzian's cone cracks).

For the fatigue tests, the first specimen of each group was tested with the initial load (~60% of the mean of the

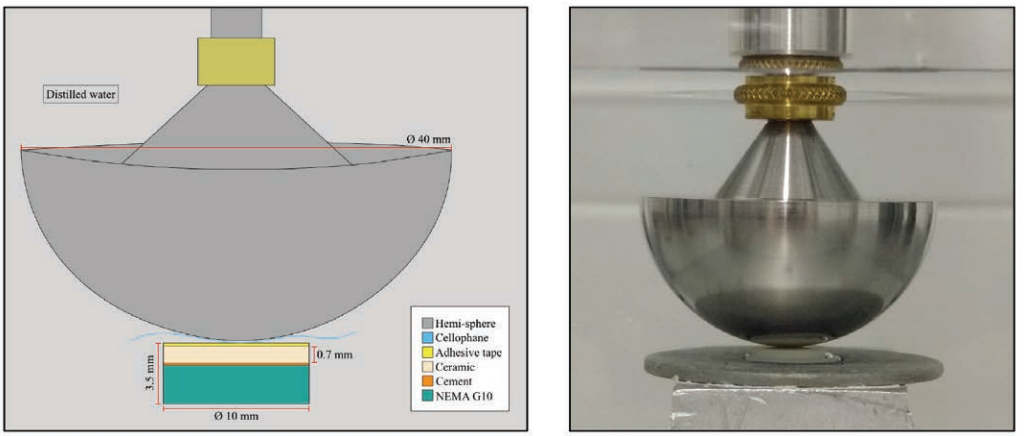

Figure 1. *Fatigue test assembly - schematic drawing of the set and photograph of the hemispheric stainless-steel piston (40 mm Ø) used to apply the load in the center of the specimens' occlusal surface, submerged in distilled water.*

load-to-failure test), and then one step-size (~5% of initial load) was added or subtracted for the next specimen depending on the previous specimen's survival (+1 step) or failure (-1 step) to the predefined cycles (250,000). The test was sequentially performed until a minimum of 15 specimens were tested after the up-and-down method had started, being, according to Collins,[37] enough to achieve an accurate fatigue measurement.

Fractographic Analysis

After fatigue testing, the fractured specimens were evaluated in a stereomicroscope (Stereo Discovery V20, Carl-Zeiss, Göttingen, Germany) to determine the crack location. The crack was marked to be cut perpendicularly in two halves in a high-precision diamond saw (Isomet 1000, Buehler). Representative specimens were selected for scanning electron microscopy analysis (Secondary Electron Detector [SE], VEGA3 Tescan, Brno-Kohoutovice, Czech Republic) to better describe their failure characteristics. The images were taken to analyze the radial crack in two different perspectives; a typical fractographic analysis of a debonded zirconia specimen after fracture (350× and 1000× magnification), and an analysis of a transversal view of the crack (250× of magnification), as mentioned above for the specimens that remained bonded after fracture.

Statistical Analysis

Two-way analysis of variance (ANOVA, IBM SPSS Statistics Program v24 for Windows, IBM Corp, New York, NY, USA; α=0.05) was used to determine the influence of the independent variables (cement and thermocycling) and their interaction (cement + thermocycling) on the dependent variable (fatigue failure load).

The mean load for fatigue failure (L_f), standard deviation (SD), and 95% confidence interval (CI) were calculated using the Dixon and Mood method,[42] which involves the maximum-likelihood estimation (overlapping confidence intervals) and assumes a normal distribution of the data,[37] as described in previous studies.[43,44]

RESULTS

Based on two-way ANOVA, a statistically significant influence was observed for the cement (p<0.001) and aging factors (p<0.001), and their interaction (cement + aging; p<0.001) on the fatigue failure load data.

The mean monotonic load-to-failure values, the parameters for fatigue tests and results, and the graphics of fatigue survival/failure patterns for each group are described in Table 3 and Figure 2.

Considering the baseline condition, the Ion cement group had the lowest fatigue load (1530.00), being statistically equal to the Self group (1570.00). After aging, the MDP-AD + RC/TC presented the highest fatigue values (1957.50), while the Ion/TC (1551.67) presented the lowest ones. Aging had no deleterious effect on fatigue loads (Table 3).

Radial crack was the fracture pattern observed for all groups and it originated from the intaglio ceramic surface. Figure 3 shows the fractographic characteristics under two perspectives; in a specimen in which the zirconia fragments separated after failure (Figures 3A and 3B), and in a transversal cut of a sectioned specimen that remained cemented after failure (Figure 3C). No cone-cracks were observed.

DISCUSSION

The present study demonstrated that the luting protocol affects the monolithic zirconia fatigue failure load, refuting the first null hypothesis, and that the aging process applied was not enough to jeopardize the mechanical behavior of the restorative assembly,

Table 3: *Mean of Monotonic Load-to-Failure Test (n=5)*[a]

Groups	Mean Monotonic Load-to-Failure (n)	Initial Load for Fatigue Test (n	Step-size Increment (n)	Mean Load for Fatigue Failure L_f (SD)	95% CI[b]	Load Decrease (%)
Ion	1998.55	1200	60	1530.00 (286.32)	1319.23 - 1740.77 B	23
Ion/TC	1773.75	1060	50	1551.67 (40.60)	1518.21 - 1585.13 c	13
Self	2382.53	1430	70	1570.00 (294.89)	1369.02 - 1770.98 AB	34
Self/TC	2181.63	1310	65	1754.17 (122.98)	1661.67 - 1846.67 b	20
PR + MDP-RC	2160.00	1300	65	1847.86 (119.10)	1764.04 - 1931.68 A	14
PR + MDP-RC/TC	2124.57	1275	65	1767.14 (58.93)	1723.05 - 1811.23 b	17
MDP-AD + RC	2172.17	1300	65	1820.00 (55.70)	1763.77 - 1876.23 A*	16
MDP-AD + RC/TC	2237.54	1340	65	1957.50 (64.48)	1905.91 - 2009.09 a*	13

Abbreviations: Ion, glass ionomer cement; Self, self-adhesive resin cement; MDP-AD + RC, MDP-containing adhesive + resin cement; PR + MDP-RC, Primer + MDP-containing resin cement; TC, thermocycling; MDP, methacryloyloxydecyl-dihydrogen-phosphate

[a]Fatigue test parameters: initial load for fatigue tests (~60% of mean monotonic load-to-failure), step-size (~5% of initial load). Fatigue results: mean load for fatigue failure (L_f)(SD) and 95% confidence interval (CI); and percentage of decreasing load comparing the mean value of monotonic load-to-failure and the mean load for fatigue failure.

[b]Statistical analysis for fatigue test - Dixon & Mood statistical method[42] (confidence intervals overlapping): different uppercase letters represent statistically significant difference for different cement systems on baseline; different lowercase letters mean statistical difference for different cement systems after thermocycling; and asterisk (*) represents statistically significant difference between baseline and aged between the same cement system.

accepting the second null hypothesis. The study results showed that bonding the air-abraded monolithic zirconia using an MDP-containing universal adhesive plus an adhesive resin cement (MDP-AD + RC system) provided the best long-term fatigue failure load results. Also, the investigated zirconia ceramic (Zenostar T) can endure high fatigue loads, even in a thin (0.7 mm) thickness, thus providing a conservative dental option for monolithic crowns in the posterior region of the mouth, being able to withstand even the highest biting forces during nocturnal bruxism, which can reach 800 N.[45]

In a recent systematic review and meta-analysis, Thammajaruk and others[46] concluded that mechanical and chemical pre-treatments are determinant on the bond strength to zirconia, particularly when MDP-containing primers are used, both with and without aging. Kern[47] reviewed and compared the best available clinical and laboratory evidence for successful bonding of dental oxide ceramic restorations and concluded that the association of air-abrasion at a moderate pressure (0.1-0.25 MPa) with the use of primers and/or resin cements containing a phosphate monomer (MDP) provides long-term durable bonding to zirconia ceramic. In the present study, better results after aging were achieved when luting the monolithic zirconia using an MDP-containing adhesive (RelyX Ultimate system).

Luting the zirconia ceramic with the resin-modified glass ionomer cement (RelyX Luting 2) led to the worst fatigue behavior after aging. The lower fatigue performance of crowns cemented with glass-ionomer systems has been shown previously.[48,49] This improvement provided by resin cements is related to their greater ability to create a strong adhesion between a dentin-like substrate and zirconia.[19] Furthermore, resin-based cements have a higher modulus of elasticity and flexural strength than ionomer-based cements, enabling a better foundation.[50]

The *in vitro* studies should simulate the aging of the materials and of the adhesive interface[18] since the restorations are exposed to different challenges in the mouth (ie, humidity, variations in temperature and pH).[51] The aging can degrade the adhesive bonding through some factors, such as cement stiffness reduction,[28] hydrolytic degradation of the materials' polymer matrix by water penetration, and fatigue of the adhesive interface due to the mismatch of linear thermal expansion coefficients (different rates of shrinkage and expansion) between bonded materials during temperature changes,[52] thereby affecting long-term success of the restoration.[18] According to Lu and

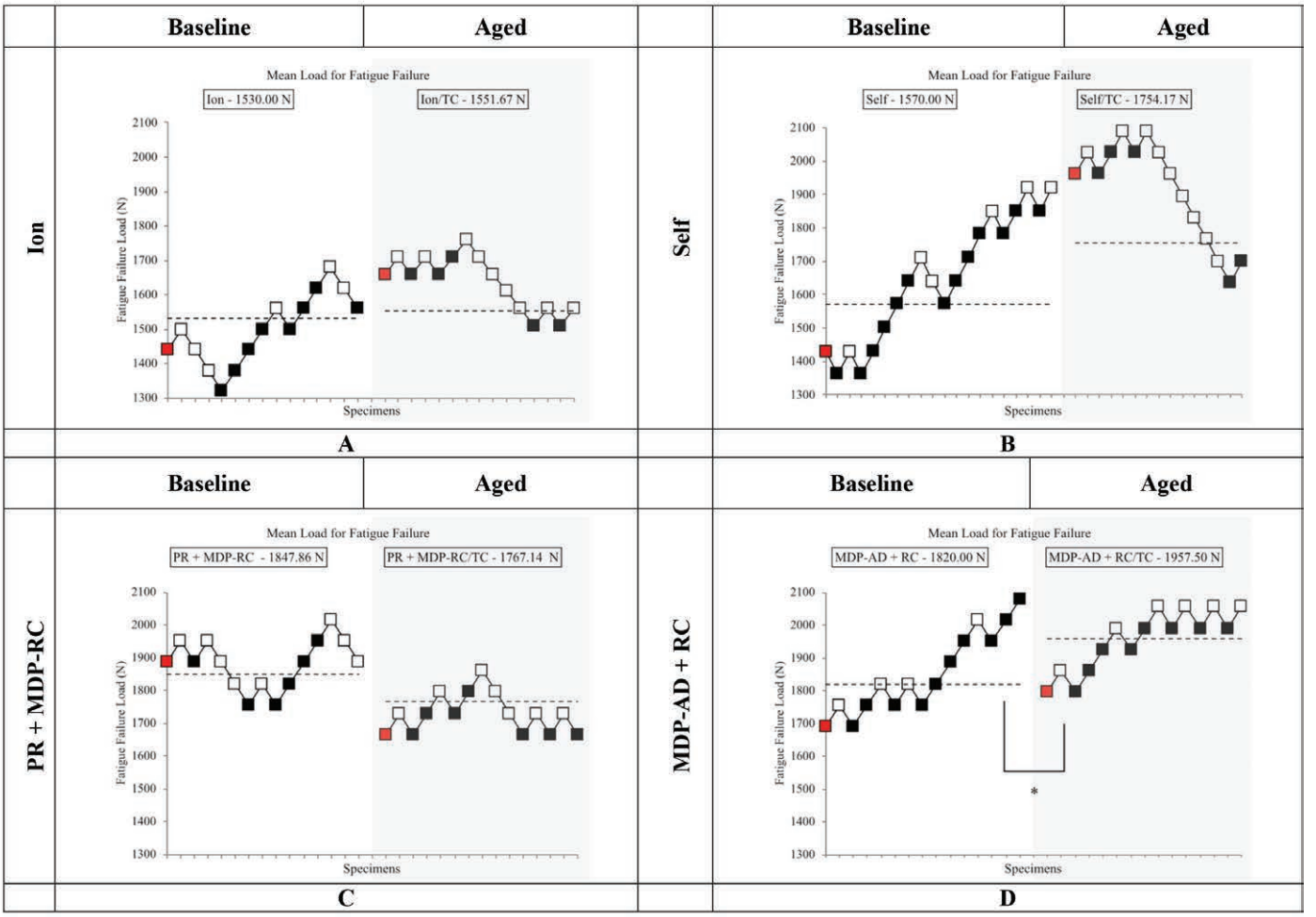

Figure 2. *Staircase survival and failure patterns for each group (n=20 or a minimum of 15 specimens tested after the up-and-down characters have been started; red marks). The horizontal dashed lines represent the mean load for fatigue failure of each group; black marks mean intact specimens and white marks mean failed ones. Asterisk (*) indicates a statistically significant difference between baseline and aged groups for the same cement system based on Table 3.*

others,[53] aging in water can degrade the bond strength and stiffness (ie, decrease of the elastic modulus) of cement agents, leading to stress redistribution in the ceramic crown, reducing its load-bearing capacity.

In the present study, we did not observe a negative effect of thermocycling on the assembly fatigue behavior, even following the number of cycles recommended by Andreatta and others[52] as being deleterious for bond strength values between ceramic material and resin cement. However, Zhao and others[54] reported that slow thermal cycling (3000 thermal cycles - 5°C/55°C, where each thermal cycle took 15 minutes) is more effective than a fast-changing temperature profile to promote the aging process of the bonding interface in materials with low thermal diffusivity (eg, zirconia). In this sense, the transfer time used in our study (only 4 seconds) could explain why thermal cycling did not deleteriously impact the fatigue load. In a previous study,[36] the aging process did affect the zirconia fatigue behavior when the zirconia was air-abraded with aluminum oxide (45 μm particle) and bonded with the Panavia F2.0 system (Kuraray, Noritake, Ukayama, Japan). That could be explained since the aging process was more aggressive in that study,[36] which applied 60 days of storage in distilled water at 37°C additionally to the thermocycling protocol (12,000 thermal cycles 5°C-55°C), which may have allowed water to penetrate and degrade the bonding interface.[52] Hygroscopic expansion is material dependent and sometimes it can exceed the amount of polymerization shrinkage, overcompensating it and leading to internal expansion stress, endangering the restoration integrity.[55]

Indeed, the aging significantly increased the fatigue failure load of the MDP-AD + RC system, and according to de Oyagüe and others,[56] this could be explained by the long carbonyl chain of the acidic functional monomer

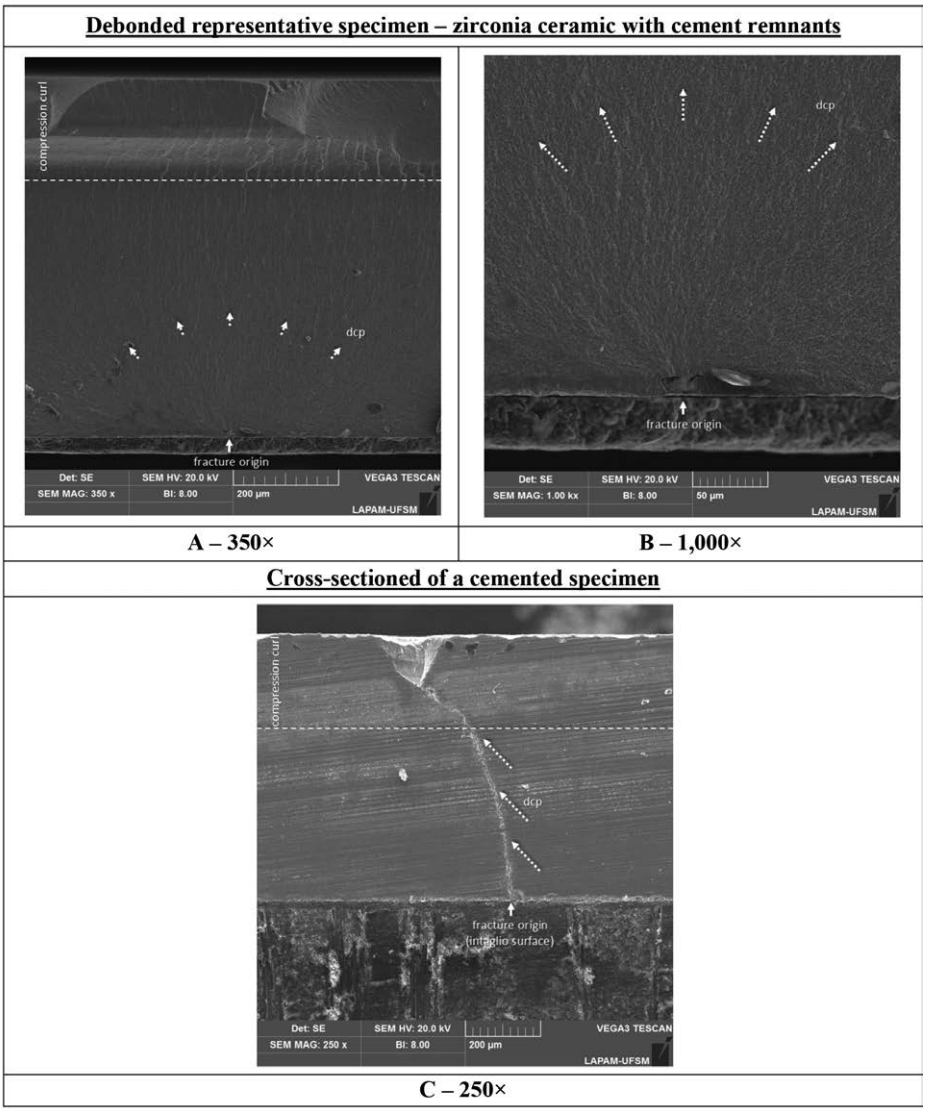

Figure 3. *SEM images showing the typical fractographic characteristics after fatigue failure under two perspectives: A and B show a specimen where ceramic fragments were separated after failure; C shows a transverse cut of a sectioned specimen that remained cemented after failure (radial crack). White arrows point to the site of fracture origin; dashed horizontal line indicates the compression curl; white dashed arrows indicate the direction of crack propagation (dcp).*[59]

present in the MDP formulation that is relatively stable to hydrolysis. From Zhao and others,[54] the presence of the methacrylate-modified polyalkenoic acid has a moisture-stabilizing effect on the Single Bond Universal Adhesive, which can explain its better behavior when subjected to aging. Furthermore, only the MDP-AD + RC system received application of an adhesive on the zirconia surface, and as adhesives have lower viscosity, they are able to better wet and fill in the ceramic surface irregularities, improving the bond strength and reducing the water penetration at the interface.[57] Still, when restorative materials absorb water, their dimensions and structural integrity may be affected, and in this case the shrinkage stress of the adhesive may be partially relieved by the water uptake, neutralizing the tensions at the adhesive interface, and better distributing the stress during loading,[55] consequently increasing the fatigue failure load of the restorative set-up.

As stated by Zhang and others,[25] a post-failure fractographic analysis can provide valuable guidance to find the fracture origin and other failure characteristics. In our study, we only found radial cracks originating from the ceramic intaglio surface (Figure 3), and no surface contact damage (Hertzian's cone cracks) was found.[38] Such findings are very important since radial cracks in the monolithic ceramic crowns can propagate and result in bulk fracture, one of the most common failure modes of all-ceramic restorations.[29]

The present study applied a simplified and standardized model, which eliminated some testing variables, and isolated the factors under study. It also followed some recommendations such as wet testing and the use of a hemispheric piston with a minimal diameter (40 mm) to create clinically sized contacts of 0.5 to 3 mm diameter (clinical wear facet size) at pressures of 5 to 890 MPa when applying realistic average maximum biting forces (100 to 700 N).[38] However, besides using a dentin-like substrate as a substitute for the human dentin, a uniaxial load was applied without clinical sliding contact, so the mechanical conditions of the oral environment were only partially reproduced.[26] Furthermore, in an attempt to reduce time spent on the fatigue testing and without significant impact on the zirconia fatigue behavior,[43] the load frequency applied (20 Hz) was much higher than a normal chewing frequency (0.94 - 2.17 Hz[58]).

CONCLUSION

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

1. The fatigue failure load of monolithic zirconia cemented to a dentin-like substrate was influenced by the luting system.
2. Aging had no damaging effect on the fatigue failure load of the monolithic zirconia specimens.
3. The use of an MDP-containing adhesive associated with a resin cement promoted better fatigue results after aging.

Acknowledgments

This work is part of the fulfillment for the requirements of the PhD degree (LFG) in the Post Graduate Program in Oral Sciences at the Faculty of Dentistry, Federal University of Santa Maria-RS, Brazil. This study was financed in part by the Brazilian Federal Agency for Coordination of Improvement of Higher Education Personnel - CAPES (Finance Code 001), and in part by the scholarship financial support at Academisch Centrum Tandheelkunde Amsterdam (ACTA) CAPES/NUFFIC program: NUFFIC Dutch Organization for Internationalization in Education; Project # 056/14; Process # 88881.145663/2017-01). The authors are grateful to Ivoclar Vivadent for providing their materials free of charge.

Conflicts of Interest

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

(Accepted 21 October 2021)

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Caries-inhibiting Effect of Microencapsulated Active Components in Pit and Fissure Sealants

S Amend • R Frankenberger • S Lücker • N Krämer

A bioactive pit and fissure sealant with microcapsules containing remineralizing agents did not show a caries inhibiting effect in an in vitro biofilm model.

http://doi.org/10.2341/20-048-L

Interfacial Bond Strength and Morphology of Sound and Caries-affected Dentin Surfaces Bonded to Two Resin-modified Glass Ionomer Cements

RM Al-Hasan • LA Al-Taee

Some resin-modified glass ionomers may enhance outcomes when used with resin composite in stress bearing areas.

http://doi.org/10.2341/21-048-L

The Effect of Different Light-curing Units and Tip Distances on the Polymerization Efficiency of Bulk-fill Materials

HNA Al Nahedh • DF Al-Senan • AS Alayad

Clinicians should exercise caution when selecting and placing resin-based bulk-filling materials using light-curing units for the restoration of deep cavities. Increased distance from the light tip has a detrimental effect on the mechanical properties of composite resin materials.

http://doi.org/10.2341/20-282-L

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Caries-inhibiting Effect of Microencapsulated Active Components in Pit and Fissure Sealants

S Amend • R Frankenberger • S Lücker • N Krämer

Clinical Relevance

A bioactive pit and fissure sealant with microcapsules containing remineralizing agents did not show a caries inhibiting effect in an *in vitro* biofilm model.

SUMMARY

Objective: The aim of the present *in vitro* study was to examine the caries-inhibiting effect of a pit and fissure sealant (PFS) containing ion-releasing microcapsules under cariogenic conditions in a biofilm artificial mouth.

Methods and Materials: Forty-eight human third molars were divided into four groups (n=12 per group). Fissures were extended with burs and sealed with experimental PFS. The four groups of specimens were treated as follows: 1) EPFS 1: EPFS (Premier Dental) of increasing viscosity, containing microcapsules loaded with remineralizing agents (calcium, phosphate, and fluoride ions); 2) US: fluoride-releasing PFS (UltraSeal XT plus, UltraDent Products, South Jordan, UT, USA); 3) EPFS 2: experimental PFS of constant viscosity containing microcapsules loaded with calcium, phosphate, and fluoride ions; and 4) FT: glass ionomer cement (GIC) (GC Fuji Triage CAPSULE WHITE glass ionomer cement, GC Europe NV, Leuven, Belgium). FT and US were used as control groups. EPFS 1 and EPFS 2 were the experimental groups. Specimens were stored in distilled water for 14 days at 37°C, subjected to 10,000 thermocycles (5°C and 55°C) and finally exposed to microbiological cycling in a Streptococcus mutans-based artificial mouth for 10 days. Replicas were made for scanning electron microscopic (SEM) evaluation and specimens were cut for fluorescence microscopy.

Results: Overall demineralization depths at the margin of Fuji Triage were significantly shallower than in the other groups (p<0.05).

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http://doi.org/10.2341/20-048-L

Overall demineralization depths adjacent to the experimental pit and fissure sealant EPFS 2 (59±15 μm) were comparable to the values of the resin-based pit and fissure sealant UltraSeal XT plus (58±10 μm, $p \geq 0.05$). SEM revealed surface roughness of the GIC-based PFS.

Conclusions: The experimental PFS with microcapsules containing active components for remineralization did not show a caries-inhibiting effect compared to a fluoride-releasing resin-based PFS. Lower demineralization depths adjacent to GIC sealants indicate an anticariogenic effect through fluoride ion release.

INTRODUCTION

In recent years, a decline in caries has been observed in many Western industrialized nations.[1,2] Epidemiological studies conducted in Germany have supported this trend of decreasing caries prevalence.[3,4] The results of the German Oral Health Studies (DMS I–V) showed a decline in the mean decayed, missing, and filled teeth count (DMF[T]) from 4.9 in 1989-1992 (DMS I and II, 13-14-year-olds) to 0.5 (DMS V, 12-year-olds) in 2014.[3] Besides the caries decline, a polarization of the caries prevalence with an increased onset of carious lesions among socially deprived children[1-3, 5-9] as well as changes in the lesion patterns have been recognized.[1,5,6,10-12] In contrast to earlier days, an increasing number of "noncavitated carious lesions" is diagnosed, especially on permanent molars in childhood and adolescence.[1]

The occlusal surfaces of the permanent molars, and especially the pits and fissures of the first permanent molars, appear to be the tooth surfaces with the highest caries susceptibility in the phase of mixed dentition.[1,7,10,13,14] To protect the permanent molars from the onset or the progression of a carious lesion, pit and fissure sealing has proven to be an effective caries-preventive treatment measure.[1,11,14] Whereas conventional pit and fissure sealants usually do not contain material-specific caries-preventive ingredients, there are other sealing materials, like fluoride-releasing pit and fissure sealants or GICs, that are supposed to have anti-bacterial and/or remineralizing effects through fluoride-release.[15] Children with a high caries risk might especially benefit from the use of these pit and fissure sealants, receiving additional protection against the occurrence of a carious lesion under cariogenic conditions.[9]

A new approach for remineralization of demineralized dental hard tissue is the incorporation of microcapsules loaded with aqueous solutions of calcium nitrate [$Ca(NO_3)_2$], sodium fluoride (NaF) and/or dipotassium phosphate (K_2HPO_4) in dental materials (rosin varnish, resin glaze, pit and fissure sealant, orthodontic cement). The microcapsules embedded in these materials are supposed to promote remineralization through a sustained, long-term release of bioavailable ions (calcium, phosphate, and fluoride ions).[16-19]

The caries-preventive properties of newly developed dental materials can be investigated *in vitro* during preliminary testing, for instance in artificial mouth models simulating the physical circumstances of the human oral cavity in a simplified way.[20] Before the launch of new products for use in clinical practice, further *in situ* and *in vivo* studies should be conducted to confirm or refute the findings of these laboratory studies.[20,21]

Against this backdrop, the aim of the present *in vitro* study was to examine the demineralization-inhibiting properties of a newly developed, resinous pit and fissure sealant containing ion-releasing microcapsules for remineralization (Premier Dental, Plymouth Meeting, PA, USA). The investigation was conducted under cariogenic conditions in a *Streptococcus mutans*-based artificial mouth model for 10 days.

The null hypothesis tested was that no differences in demineralization-inhibiting effects would be measurable among the four pit and fissure sealants after exposing them to a microbiological load in an artificial mouth model for 10 days. Measurements taken included gap width, demineralization depth, substance loss, and overall demineralization depth (as the sum of the two parameters mentioned above) at the sealing margin and at a 500-μm distance from the margin. Furthermore, it was hypothesized that differences in the surface quality of the pit and fissure sealants would not be discernable by investigation under SEM.

The alternative hypothesis was that the four pit and fissure sealants subjected to 10 days of cariogenic challenge in an artificial mouth model would exhibit different demineralization-inhibiting effects through ion release adjacent to the sealing margin and at a 500-μm distance from the margin. Additionally, it was hypothesized that the microbiological load would lead to qualitative differences in sealant surface quality.

METHODS AND MATERIALS

Preparation of Specimens and Fissure Sealing

Forty-eight caries-free human third molars that had either been extracted or osteotomized for therapeutic reasons were used in this study. After extraction or osteotomy, the teeth were stored in 0.5% chloramine-T solution (Chloramin T Trihydrat, Carl Roth GmbH & Co KG, Karlsruhe, Germany) at 4°C for up to

twenty-eight days. The teeth were randomly assigned to four groups (n/group=12). The specimens were then cleaned by air-blasting (PROPHYflex 3, KaVo Dental GmbH, Biberach/Riss, Germany; powder: Clinpro Glycine Prophy Powder, 3M Oral Care, Germany, Neuss, Germany) and rinsed with water spray, and the fissures were prepared using a Fissurotomy-bur (type "original", Fissurotomy burs, SS White Bur, distributor: *atec* Dental GmbH, Ebringen, Germany). After air-drying of the occlusal surfaces, the following pit and fissure sealants were used to seal the prepared fissures (Table 1):

Group 1 (EPFS 1)—Experimental calcium-, phosphate-, and fluoride-releasing resin-based pit and fissure sealant containing microcapsules loaded with remineralizing agents (Batch No 1, proprietary composition; Premier Dental)

Group 2 (US)—UltraSeal XT *plus* (fluoride-releasing resin-based pit and fissure sealant; Ultradent Products)

Group 3 (EPFS 2)—Experimental calcium-, phosphate-, and fluoride-releasing resin-based pit and fissure sealant containing microcapsules loaded with remineralizing agents (Batch No 2, proprietary composition; Premier Dental)

Group 4 (FT)—GC Fuji Triage CAPSULE WHITE (glass ionomer cement; GC)

In Group 1 (EPFS 1), Group 2 (US), and Group 3 (EPFS 2), 35 % phosphoric acid in gel form (Ultra-Etch, Ultradent Products) was applied on enamel for 30 seconds and if an exposure of dentin was visible, the dentin was etched for 15 seconds. Then, Ultra-Etch (Ultradent Products) was rinsed off with water spray for minimally 10 seconds, followed by air-drying. The pit and fissure sealants were applied in three layers with a gentle dispersion of each layer to avoid void formation and afterwards polymerized for 30 seconds (Bluephase; Ivoclar Vivadent AG, Schaan, Liechtenstein). The light intensity of the LED curing device was monitored regularly with a radiometer (CURE RITE, Dentsply Caulk, Milford, DE, USA) to ensure a light output of at least 1200 mW/cm^2. Finally, the oxygen inhibition layer was removed by wiping the pit and fissure sealant surfaces with a foam pellet (Pele Tim No 2, Voco GmbH, Cuxhaven, Germany).

Table 1: *Pit and Fissure Sealants Under Investigation in the Present Study*

Material (Group; Manufacturer)	Classification	Composition	LOT Number
ENAMEL LOC (EPFS 1; Premier Dental, Plymouth Meeting, PA, USA)	Experimental microencapsulated resin-based PFS	Proprietary composition: ≤40 % monomers, ≤60 % barium aluminoborosilicate glass, ≤2.5 % photo initiator, ≤2% bioactive donor containing Ca^{2+} ions, ≤2% bioactive donor containing (PO4)3- ions, ≤2% bioactive donor containing F- ions, ≤2 % other	1811SMC
UltraSeal XT plus opaque white (US; Ultradent Products Inc, South Jordan, UT, USA)	Fluoride-containing resin-based PFS	>10%-≤25% TEGDMA, >2.5%-≤10% DUDMA, >2.5%-≤10% aluminum oxide, ≤2.5% HEMA, ≤2.5% amine methacrylate, ≤2.5% organophosphine oxide, 0.1% sodium monofluorophosphate	BBWLW
BIOACTIVE Premier Sealant (EPFS 2; Premier Dental)	Experimental microencapsulated resin-based PFS	Proprietary composition: ≤40% monomers, ≤60% Barium aluminoborosilicate glass, ≤2.5% photo initiator, ≤2% bioactive donor containing Ca2+ ions, ≤2% bioactive donor containing (PO4)3- ions, ≤2% bioactive donor containing F- ions, ≤2% other	5100PSMC
GC Fuji Triage CAPSULE WHITE (FT; GC Europe NV, Leuven, Belgium)	Glass ionomer cement	Powder: Fluoroaluminosilicate glass Liquid: Polyacrylic acid, polybasic carboxylic acid, distilled water	1512171

Abbreviations: Ca^{2+}, calcium ion; F-, fluoride ion; PFS, pit and fissure sealant; TEGDMA, triethylene glycol dimethacrylate; DUDMA, diurethane dimetharylate; HEMA, 2-hydroxyethyl methacrylate; (PO4)3-, phosphate ion.

The application of Fuji Triage (GC Europe NV) in Group 4 was done in one layer, after the product was activated and mixed according to the manufacturer's specifications. Heliobond (Ivoclar Vivadent) was applied on the pit and fissure sealant surfaces using a Microbrush (Microbrush International, Grafton, WI, USA), and polymerized for 30 seconds (Bluephase; Ivoclar Vivadent) to protect the glass ionomer cement against humidity and dehydration during the setting reaction. Since it took about 24 hours until the setting reaction of the glass ionomer cement was completed, the specimens were first stored in distilled water for 24 hours at 37°C, and then further processing was performed.

Following sealant application, the occlusal surfaces were ground until the cusps were flattened and polished (BUEHLER Beta GRINDER - POLISHER, sandpaper: BUEHLER CarbiMet Grit 600 [P1200 and P4000], ITW Test & Measurement GmbH, Düsseldorf, Germany) in order to attain a smooth surface at the pit and fissure sealant-enamel interface. Thereafter, the specimens were stored in distilled water for 14 days at 37°C (Incubator Type B20, Heraeus Holding GmbH, Hanau, Germany), and subjected to 10,000 thermocycles (5°C and 55°C, dwell time 15 seconds; TCS 30, Syndicad, Munich, Germany). After the apical thirds of the roots were cut off, the teeth were mounted on chewing simulator holders (Festo AG & Co KG, Denkendorf, Germany) with glue wax (Chemical Dental Laboratory, Oppermann-Schwedler, Bonn, Germany), followed by disinfection in 70% ethanol for 120 minutes. The specimens were then exposed to a microbiological load in an automated, *S mutans*-based artificial mouth model for 10 days with a total of 4 hours of demineralization/day.

Artificial Mouth Model

The computer-controlled artificial mouth model was composed of a reaction chamber (300-4100 Reusable Filter Holder with Receiver, Thermo Scientific Nalgene Labware, Rochester, NY, USA) containing a Teflon holder (Bretthauer GmbH, Dillenburg, Germany) to attach the teeth and a pH-measuring electrode (SI Analytics Electrode N1048 1M – DIN – ID, SI Analytics GmbH, Mainz, Germany; Schott Instruments Lab 870, Schott AG, Mainz, Germany; MultiLab pilot v.4.7.2, WTW GmbH, Weilheim, Germany), an Erlenmeyer flask acting as a bacterial reservoir (Schott AG), two 20 L bottles (Schott AG), one for the nutrient medium and the other for artificial saliva, and a 10 L bottle (Thermo Scientific Nalgene) for liquid waste. Computer-controlled peristaltic pumps (Cyclo II, Carl Roth GmbH & Co KG; LeC, Conrad Electronic SE, Hirschau, Germany) transported the liquids within the experimental setup. To ensure a temperature of 37°C during test-efforts, the artificial mouth model was built inside an incubator (IPS Memmert, Memmert GmbH & Co KG, Schwabach, Germany). All components of the artificial mouth model except the pH measuring electrode could be disassembled and sterilized at 121°C and 2 bars for 15 minutes (autoclave VX-75, Systec GmbH, Linden, Germany). After calibration, the pH measuring electrode was disinfected with 70% ethanol and rinsed with distilled water. To prevent undesirable contamination, the specimens were placed in the Teflon holder inside the reaction chamber under a clean bench (Thermo Fisher Scientific Inc, Waltham, MA, USA). The pH measuring electrode was also attached to the reaction chamber under the clean bench before every episode of testing.

To simulate exposure of specimens to intermittent demineralization and remineralization phases within the artificial mouth model, a nutrient medium, a bacterial strain, and artificial saliva were needed. The nutrient medium utilized for bacterial proliferation within the artificial mouth model was Schaedler Broth (BD, BBL Schaedler Broth, Becton Dickinson and Company, Sparks, MD, USA), which was used according to manufacturer's specifications (Table 2). To simulate the remineralizing effects of human saliva within the oral cavity, a remineralizing solution as described by Zampatti and others was used as artificial saliva (pH 7; Table 3).[22]

Freeze-dried *S mutans* (DSM No: 20523, Leibniz-Institute DSMZ GmbH, Braunschweig, Germany) was stored in glycerin cultures at −80°C. After thawing, bacterial cultures were cultivated on Columbia blood

Table 2: *Ingredients of 28.4 g Schaedler Broth (BD, BBL Schaedler Broth, Becton Dickinson and Company, Sparks, MD, USA)*[a]

Ingredient	Weight (g)
Pancreatic digest of casein	8.10
Peptic digest of animal tissue	2.50
Papaic digest of soybean meal	1.00
Dextrose	5.82
Yeast extract	5.00
Sodium chloride	1.70
Dipotassium phosphate	0.82
Hemin	0.01
L-cystine	0.40
TRIS (hydroxymethyl)aminomethane	3.00

[a]*According to manufacturer's specifications for dissolution in 1 L distilled water.*

Table 3: *Ingredients Dissolved in 20 L Distilled Water for the Production of Artificial Saliva Based on the Description by Zampatti and others*[22]

Ingredient	Weight (g/20 l)	Manufacturer
NaCl	11.92	Merck KGaA, Darmstadt, Germany
KCl	15.96	
$MgCl_2$ x $6H_2O$	1.18	
$CaCl_2$ x $2H_2O$	3.18	
KH_2PO_4	5.99	
K_2HPO_4	15.99	
$NaHCO_3$	4.00	
Trypticase	5.00	Becton, Dickinson and Company
Yeast extract	5.00	

Abbreviations: NaCl, sodium chloride; KCl, potassium chloride; $MgCl_2$ x $6H_2O$, magnesium chloride hexahydrate; $CaCl_2$ x $2H_2O$, calcium chloride dihydrate; KH_2PO_4, monopotassium phosphate; K_2HPO_4, dipotassium phosphate; $NaHCO_3$, sodium hydrogencarbonate.

agar (sheep blood, OXOID AGS, Oxoid Limited, Basingstoke, Hampshire, UK) for 48 hours at 37°C under aerobic conditions. Overnight cultures of *S mutans* dissolved in Schaedler Broth (12 hour incubation, 37°C, aerobic conditions) were then produced and diluted 1:10 (8 hour incubation, 37°C, aerobic conditions). After the optical density of the 1:10 diluted overnight culture, which was targeted to be ~1 at 600 nm, was controlled, the inoculation procedure was conducted by pipetting 1 ml of the bacterial culture into the bacterial reservoir. Following proliferation of *S mutans* within the bacterial reservoir for 8 hours at 37°C, the microbiological load was begun. While the specimen demineralization was caused by acidogenic *S mutans* producing organic acids through sugar metabolism during glycolysis, remineralization was simulated by exposure to artificial saliva. During this microbiological stress protocol, the demineralization phases (duration 1 hour, pH Ø 4.2-4.3) and remineralization phases (duration 5 hours, pH Ø 7.0) alternated for 10 days, so that a total of 4 hours of demineralization and 20 hours of remineralization was achieved per day.

To control the microbiological viability of *S mutans* and the absence of bacterial contamination during the experimental procedures, samples of the bacterial solutions were cultivated on BHI agar plates (BBL, Becton, Dickinson) for 48 hours at 37°C under aerobic conditions.

Following the microbiological loading, specimens were disinfected by storage in 70% ethanol for 3 minutes. In the next step, the specimens were cut bucco-orally in slices of 1-mm thickness by means of a microtome (IsoMet 1000 Precision Saw, Buehler, ITW Test & Measurement GmbH).

Furthermore, two sets of epoxy replicas of each specimen occlusal surface were produced by taking impressions with a double-mix technique using a vinylpolysiloxane (Panasil Putty, Panasil initial contact Light, Kettenbach GmbH & Co KG, Rosbach, Germany) and casting them with AlphaDie MF (Schuetz Dental GmbH, Rosbach, Germany) to optimally visualize the quality of the enamel-pit and fissure sealant interface. The first set of replicas was manufactured after thermocycling, and the second one after microbiological loading.

In addition, fracture specimens of each fissure sealant were prepared by producing beams and fracturing those into halves to assess the surface quality.

Data Evaluation

During fluorescence microscopy evaluation, the demineralization depth, substance loss, and overall demineralization depth as the sum of the previous two parameters were evaluated for the enamel at the fissure sealant margin and at a point 500 µm from it (AZ 100M, Nikon, Tokyo, Japan; FITC-filter: 450-490 nm, spacing: 515-565 nm; NIS Elements for Windows XP, 0.9 µm/px; Figure 1). If a gap was detectable at the fissure sealant-enamel interface, the gap width was assessed. These values were measured adjacent to the fissure sealing on both sides of each tooth slice.

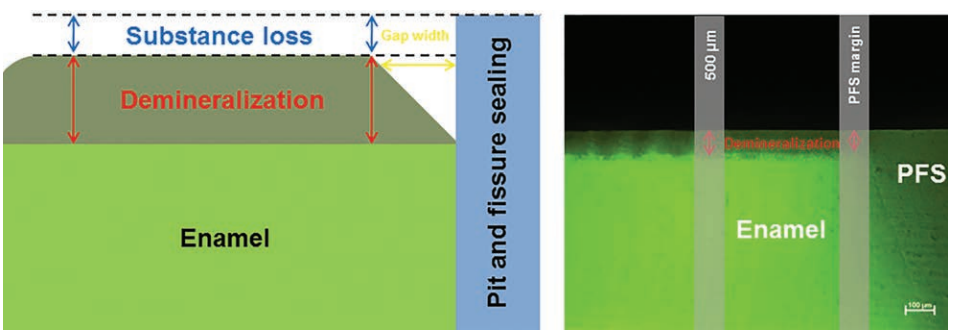

Figure 1. *Fluorescence microscopic evaluation of gap width, demineralization depth, and substance loss at pit and fissure sealing margin and in 500 µm distance from margin. PFS: Pit and fissure sealant.*

Additionally, the two sets of epoxy replicas were examined with an SEM (Amray Turbo 1610, Amray Inc, Bedford, MA, USA; 10-kV acceleration voltage, 200× magnification) to investigate the quality of the fissure sealant margins. To best investigate, SEM photographs were taken of the fracture preparation surfaces at 500×, 1000×, and 1500× magnifications to examine surface roughness.

One additional specimen of each group was prepared for energy-dispersive X-ray analysis (Quantax spectrometer, X-Flash 5010, Bruker Nano GmbH, Berlin, Germany) with an SEM (JSM-6510, JEOL, Tokyo, Japan) to obtain information about the chemical composition of the pit and fissure sealants. Specimens were attached to aluminum sample trays with a conductive carbon cement (Leit-C, Plano GmbH, Wetzlar, Germany) and sputter-coated with gold (JFC-1200 fine coater, Tokyo, Japan). SEM images of the specimens were captured at 2000× magnification (acceleration voltage: 15 kV) and energy-dispersive X-ray (EDX) analyses were conducted with unchanged settings at count rates of 1 kilocount per second (kcps).

IBM SPSS Statistics 26 (SPSS Inc, Chicago, IL, USA) was used for statistical data evaluation. Normal distribution of the measured values was checked by means of the Kolmogorov-Smirnov test. One-way analysis of variance was performed to verify the existence of statistically significant differences between the fissure sealants used in this study (ANOVA, mod LSD, α=0.05). Homogeneity of variance was found to exist in cases in which ANOVA was calculated (Levene test). The Mann-Whitney test was applied for statistical evaluation in the remaining cases with sensitive disturbances of the normal distribution of data. The significance level was set at α=0.05.

RESULTS

Application of the New Pit and Fissure Sealant

During pit and fissure sealing, EPFS 1 showed viscosity fluctuations, with the sealant being of low viscosity at the beginning of the application and becoming more viscous as the syringe was emptied. These viscosity fluctuations were not observed for the enhanced pit and fissure sealant EPFS 2.

Fluorescence Microscopy

After the 10-day exposure of the specimens to the microbiological load in the artificial mouth model, the overall demineralization depths at the sealant margins for FT (Group 4) were significantly smaller than those of the other groups (ANOVA, mod LSD, $p<0.05$; Table 4, Figures 2-5). The overall demineralization depths adjacent to the new, experimental pit and fissure sealant EPFS 2 (59±15 µm; Group 3; Figure 4) were comparable to the values of US measured at the sealant margin (58±10 µm; Group 2, Figure 3; ANOVA, mod LSD, $p\geq0.05$).

At the point 500 µm from the sealant margin, no statistically significant differences in the overall demineralization depths between the groups were detectable (Mann-Whitney test, $p\geq0.05$, Figures 2-5).

Scanning Electron Microscopy

Scanning electron microscopic images taken from representative replicas of all four groups showed the impact of the sealant-biofilm interaction after the 10-day microbiological load. On the one hand, sealant surfaces of the resin-based pit and fissure sealants (EPFS 1, US, EPFS 2) showed smooth surface structures with gap-

Table 4, *Mean values and Standard Deviation (SD) of Gap Width and Demineralization (µm) at Pit and Fissure Sealant*

Demineralization µm [SD]	**Enamel[a]**						
	GW	**D-M**	**SL-M**	**OD-M**	**D-500**	**SL-500**	**OD-500**
EPFS 1 (Batch No 1)	0(0) a	69(13) A	2(2) a	70(12) A	77(13) a	3(3) a	80(12) a
UltraSeal XT *plus*	0(0) a	58(10) A	0(0) b	58(10) B	75(6) a	0(0) b	75(6) a
EPFS 2 (Batch No 2)	0(0) a	58(15) A	1(1) ab	59(15) B	74(13) a	2(4) ab	76(15) a
GC Fuji Triage (white)	2(5) a	26(16) B	0(0) b	26(16) C	72(12) a	0(1) b	72(12) a

Abbreviations, EPFS, experimental pit and fissure sealant, GW, gap width, D-M, demineralization at sealing margin, SL-M, substance loss at sealing margin, OD-M, overall demineralization at sealing margin, D-500, demineralization in 500 µm distance from sealing margin, SL-500, substance loss at 500 µm distance from sealing margin, OD-500, overall demineralization at 500 µm distance from sealing margin.

[a]Enamel interface and at a 500 µm distance from sealant margin depending on the sealant used after microbiological load in the artificial mouth model for 10 d. Statistically significant differences between the pit and fissure sealants are marked by different uppercase letters (ANOVA, mod LSD, p<0.05). Different lowercase letters label statistically significant distinctions in cases of deviation from normal distribution (Mann-Whitney test, p<0.05).

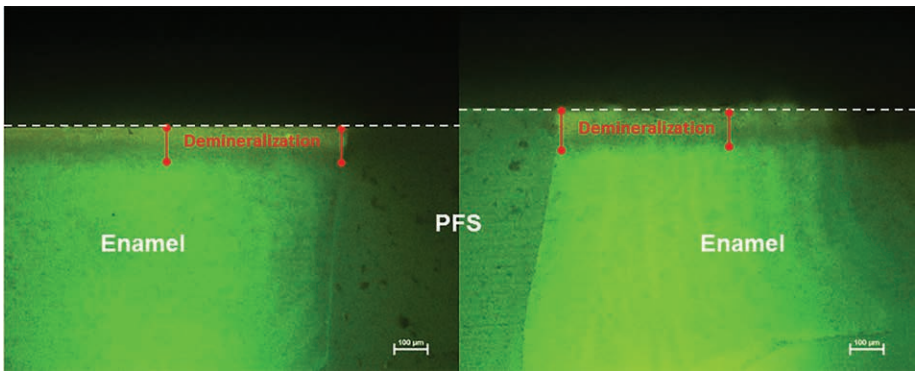

Figure 2. *Fluorescence microscopic evaluation of demineralization depth at pit and fissure sealant margin and at 500 µm distance from sealing margin (Tooth 6; experimental pit and fissure sealant No 1). PFS: pit and fissure sealant.*

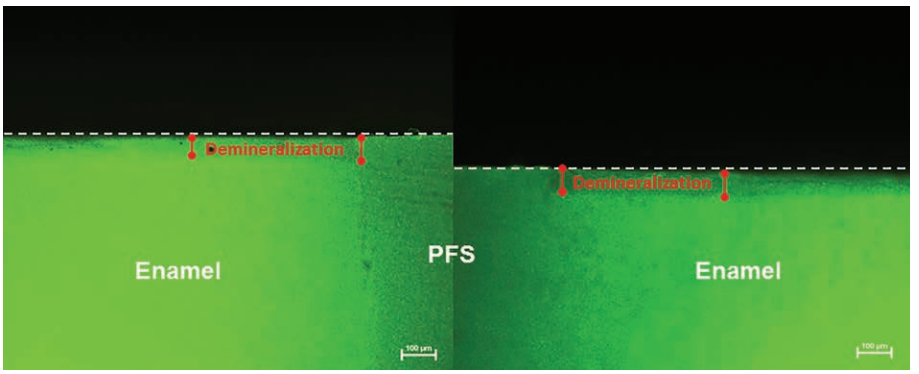

Figure 3. *Fluorescence microscopic evaluation of demineralization depth at pit and fissure sealant margin and at 500 µm distance from sealant margin (Tooth 19; UltraSeal XT plus). PFS: pit and fissure sealant.*

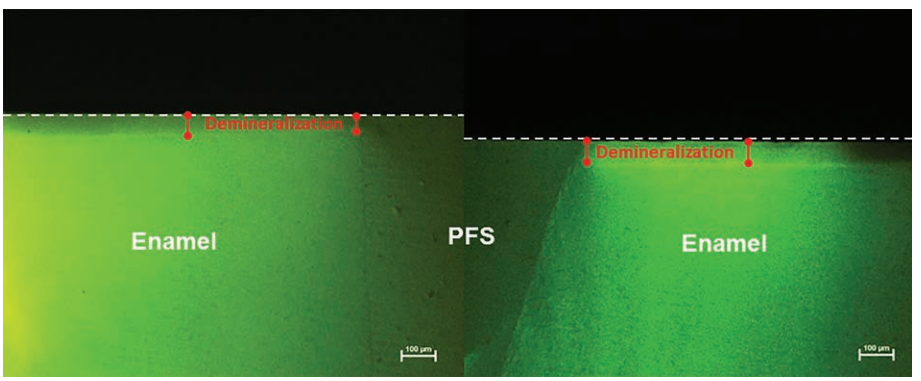

Figure 4. *Fluorescence microscopic evaluation of demineralization depth at pit and fissure sealant margin and at 500 µm distance from sealant margin (Tooth 40; experimental pit and fissure sealant No 2). PFS: pit and fissure sealant.*

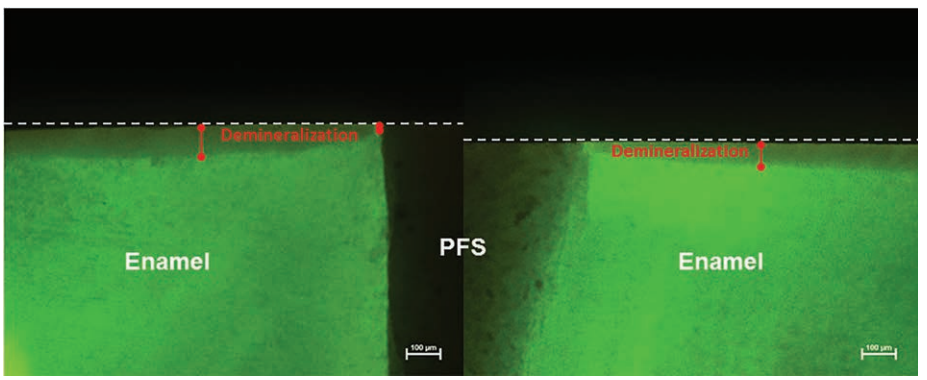

Figure 5. *Fluorescence microscopic evaluation of demineralization depth at pit and fissure sealing margin and at 500 µm distance from sealant margin (Tooth 33; Fuji Triage)PFS: pit and fissure sealing.*

free margins after biodegradation. On the other hand, images of FT surfaces (group 4) revealed an increased surface roughness with cracks and air voids not seen in the other groups (Figures 6 and 7). Additionally, the surface of the fracture preparations produced with FT appeared to be rougher than those of the other groups (Figure 8).

Energy-dispersive X-ray Analysis

SEM images and spectra of the EDX analyses are presented in Figure 9. EPFS 1 and EPFS 2 showed granular particles, which were interpreted as microcapsules. In contrast, US exhibited a smooth surface layer. The surface of FT revealed cracks and surface roughness. The comparison of EDX spectra showed that fluorine detection was lowest for US, followed by EPFS 1, EPFS 2, and FT. Calcium elements were detected in EPFS 1 but not in EPFS 2. In contrast, the spectrum of specimen EPFS 2 showed phosphorus elements; these were undetectable in the spectrum of specimen EPFS 1.

DISCUSSION

The present study was designed to investigate potential demineralization-inhibiting effects of a new, experimental pit and fissure sealant after a 10-day exposure to a microbiological load in a completely automated, *S mutans*-based, artificial mouth model. To assess this effect, the demineralization depths adjacent to the four different pit and fissure sealants were measured during fluorescence microscopy evaluation, and visible changes in the surface quality between two points in time (after thermocycling and after microbiological loading) were checked under SEM.

Recently developed dental materials with microencapsulated remineralizing agents have shown a potential for the sustained, long-term release of calcium, phosphate, and/or fluoride ions[16,17] and an ability to remineralize artificial carious lesions *in vitro*.[16] Polyurethane-based microcapsules containing aqueous solutions of $Ca(NO_3)_2$, NaF, or K_2HPO_4 have been found to be capable of releasing ions [Ca^{2+}, F^-, or $(PO_4)^{3-}$], whose presence is an important prerequisite for remineralization of demineralized dental hard tissue.[16-19] Burbank and others examined the ion release of a pit and fissure sealant containing microencapsulated 5 molar (M) $Ca(NO_3)_2$, 0.8 M NaF, and 6.0 M K_2HPO_4 in aqueous solutions separately or in combination.[16] The authors reported a sustained release of Ca^{2+}, F^-, and $(PO_4)^{3-}$ ions for about 180 days. Moreover, the fluoride uptake into artificially demineralized bovine enamel was significantly higher when specimens were exposed to a pit and fissure sealant containing a mixture of these remineralizing molecules compared to a microcapsule-free sealant. After storage in nanopure water for 90 days, the fluoride content of the bovine enamel had increased from 1.7 ± 0.7 µg F/g to 190 ± 137 µg F/g when the microencapsulated pit and fissure sealant containing 2

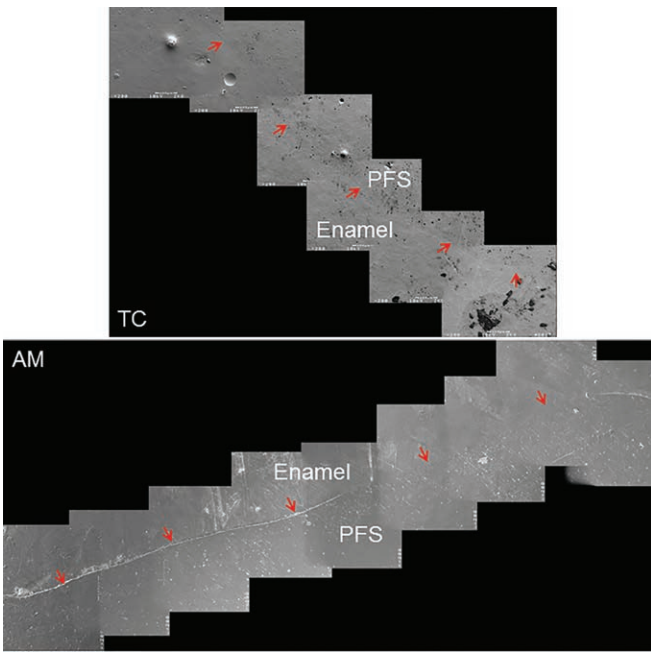

Figure 6. Example of overlapped SEM pictures taken from the replicas of tooth 26 (experimental pit and fissure sealant No 2) to visualize the quality of the pit and fissure sealant margin after thermocycling (TC) and after microbiological load in the artificial mouth model (AM) Red arrows mark the enamel-pit and fissure sealant interface. PFS: pit and fissure sealing.

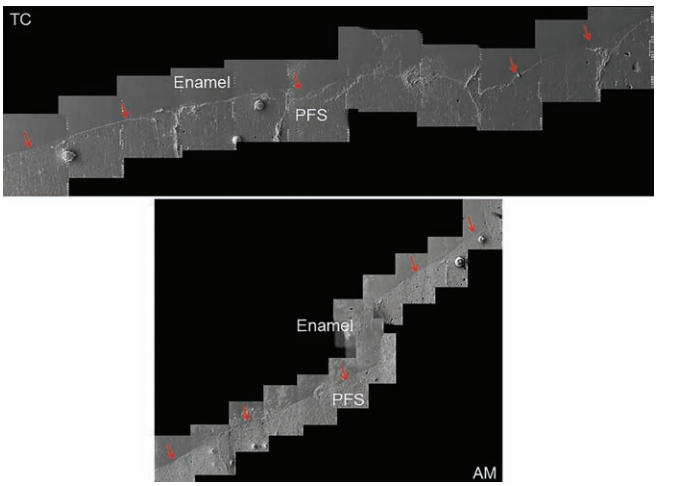

Figure 7. Example of overlapped SEM pictures taken from the replicas of tooth 32 (Fuji Triage) to visualize the quality of the pit and fissure sealing margin after thermocycling (TC) and after microbiological load in the artificial mouth model (AM). Red arrows mark the enamel-pit and fissure sealant interface. PFS: pit and fissure sealant.

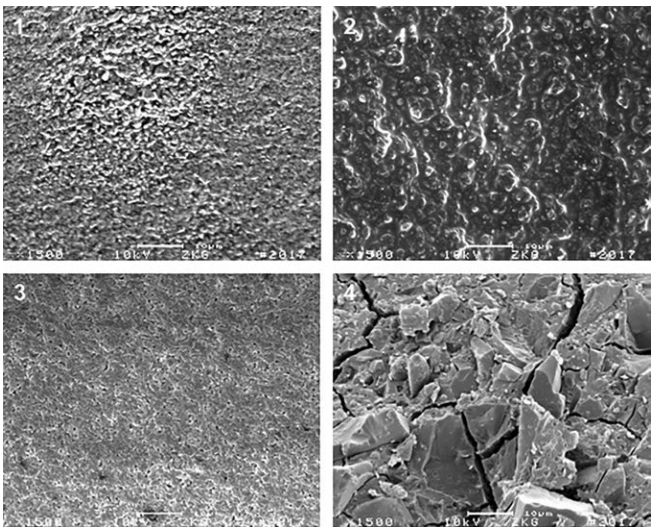

Figure 8. SEM evaluation of fracture preparations of (A) EPFS 1; (B) US; (C) EPFS 2; (D) FT. The FT surface shows an increased roughness with crack formation. EPFS: experimental pit and fissure sealant; US: UltraSeal XT plus; FT: Fuji Triage.

w/w% $Ca(NO_3)_2$, 2 w/w% NaF, and 1 w/w% K_2HPO_4 was used.[16] To our knowledge, the ability of pit and fissure sealant containing these microencapsulated remineralizing agents to prevent demineralization under cariogenic conditions has not been tested before.

Caries-free human third molars (ICDAS II Code 0) were chosen for this study,[23,24] and before sealant application the pits and fissures were prepared with a Fissurotomy-bur, which aimed to achieve the even cavity wall necessary for correct alignment of the specimens during fluorescence microscopic analysis and for a standardized evaluation. In addition, the preparation enabled the definite exclusion of "hidden caries", dentin caries hidden under an almost intact surface.[25] Not least because of the preparation, a higher amount of sealing material could be applied in the pit and fissure system, which was advantageous since a slight reduction of the sealing material had to be expected during grinding of the occlusal surfaces. The occlusal surfaces of the specimens had to be ground and polished to obtain plain and smooth surfaces and guarantee a comparable bacterial adhesion. It has to be mentioned that the cavity preparation was solely performed for experimental purposes in the present study. In daily practice, neither a cavity preparation prior to pit and fissure sealing, nor flattening of cusps should be performed in caries-free teeth.

Resin-based pit and fissure sealants usually contain a small amount of filler (6.5-54.3 wt%), preserving a low viscosity and a low Vickers microhardness of the sealing material.[26] For large pit and fissure sealants, the inferior mechanical properties of the material are disadvantageous and flowable composites should be used instead.[26] As there was no mechanical stress in terms of chewing or clenching operating on the specimens during the microbiological loading in the artificial mouth model, the pit and fissure sealants were used to seal the prepared pits and fissures in this study. The resin-based pit and fissure sealants were applied in three layers to compensate for polymerization shrinkage and to avoid void formations. It must be remembered that this method was only performed for experimental purposes and does not reflect daily clinical practice, where pit and fissure sealants are applied in one layer,[1] and where the use of flowable composites is recommended after fissurotomy because of their more favorable mechanical properties.[26]

In the present study, one fluoride-releasing resin-based pit and fissure sealant (UltraSeal XT *plus*) and one GIC (GC Fuji Triage) were chosen as comparators to the experimental pit and fissure sealant. All of these pit and fissure sealants are categorized as ion-releasing, which is a limitation of the present study. The microbiological load in the artificial mouth model simulated highly cariogenic conditions that can be expected in children with a high caries risk. In daily practice, the material of choice in these cases is a fluoride-releasing rather than a conventional pit and fissure sealant as it provides the benefit of a caries-protective effect from fluoride release in addition to its function as a physical barrier within the pit and fissure system.[27] It has been shown by Alsaffar and others that the mean mineral loss of enamel adjacent to US was comparable to the values of the control group sealed with a conventional, fluoride-free pit and fissure sealant after 20 days of demineralization with lactic acid gel.[27] Delben and others compared the anticariogenic effect of US to four other fluoride- and/or amorphous calcium phosphate-containing pit and fissure sealants. After pH-cycling, specimens of the US group exhibited the lowest surface hardness, the highest mineral loss, and a subsurface lesion formation within adjacent enamel.[28] Although US contains fluoride ions, the results of *in vitro* studies indicate that it behaves like a conventional pit and fissure sealant under simulated cariogenic conditions, which may be attributed to its comparatively low fluoride ion release.[29]

The glass ionomer cement, GC Fuji Triage, was used as a positive control in the present study. A demineralization-protective effect of this GIC was observed by Alsaffar and others, who demonstrated decreased mineral loss of adjacent enamel after 20 days of demineralization *in vitro*.[27] Additionally, Poggio and others found a significantly higher fluoride release (1.1±0.3 - 8.0±0.6 ppm F^-) for FT compared to

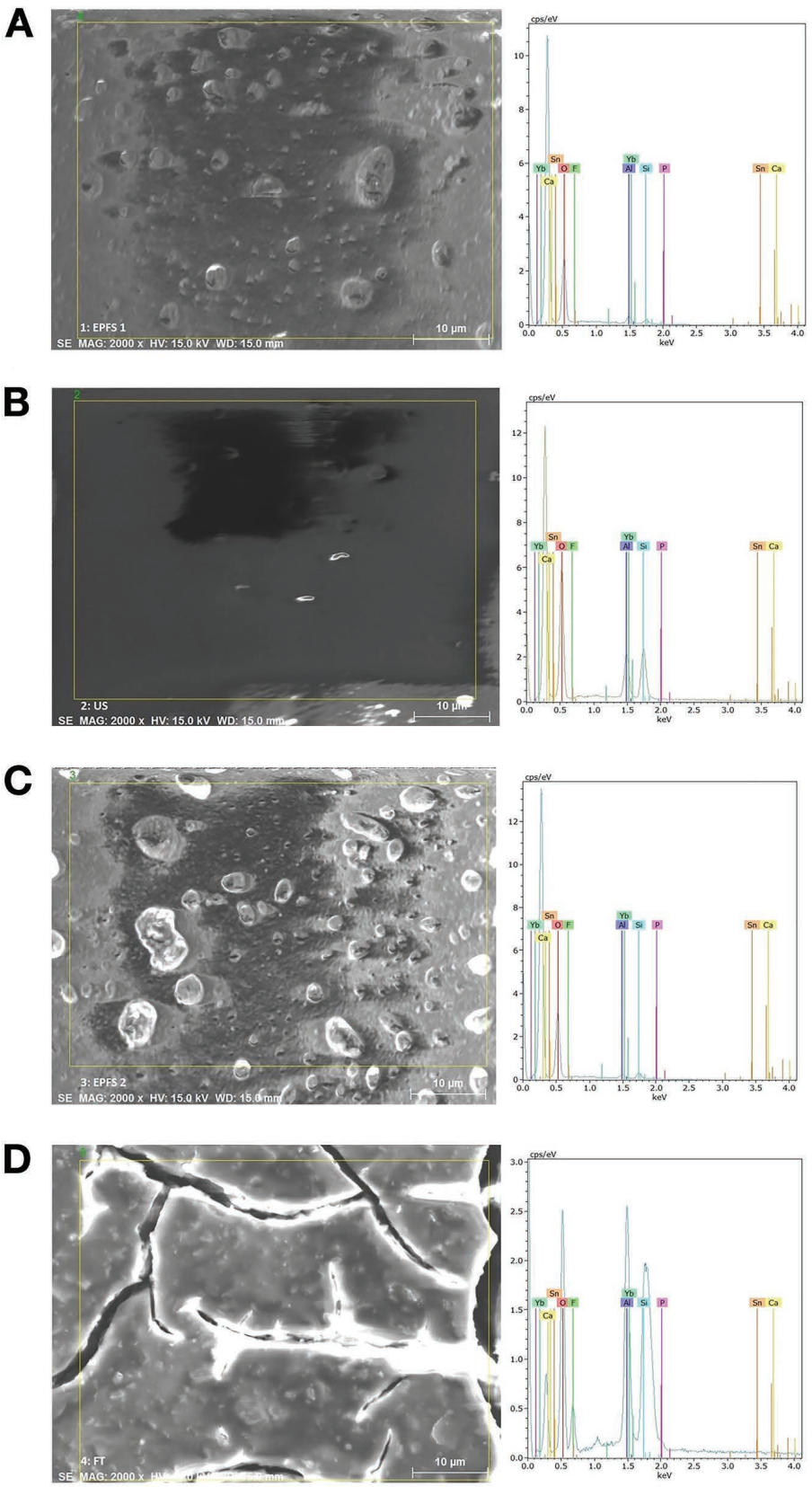

Figure 9. SEM images and EDX spectra of the pit and fissure sealants: (A): EPFS 1; (B): US; (C): EPFS 2; (D): FT. EPFS: experimental pit and fissure sealant; US: UltraSeal XT plus; FT: Fuji Triage.

a conventional and a fluoride-releasing pit and fissure sealant over a period of 49 days.[30] Clinical trials support the caries-preventive effect of FT when this GIC of low viscosity and high fluoride release is used as a pit and fissure sealant.[31-33]

The main difference between the two batches of the experimental pit and fissure sealants was related to the sealants' flow properties. During the application by syringe of Batch No 1, the material showed an increasing viscosity as the syringe was emptied. The viscosity fluctuations observed in Batch No 1 (EPFS 1) may be attributable to an agglomeration phenomenon of the microcapsules or a separation of the microcapsules from the matrix. Batch No 2 (EPFS 2) showed a uniform viscosity, which enabled good sealant application. The EDX spectra of EPFS 1 and EPFS 2 indicated differences in chemical composition, as calcium elements were only detected in EPFS 1 and phosphorus elements solely in EPFS 2 (Figure 9). Due to the proprietary nature of the compositions, more information about differences between the two batches of the experimental sealant could not be obtained from the manufacturer. In a preliminary microleakage test, the experimental pit and fissure sealant exhibited a sealing ability and a formation of voids that were both comparable to those of Helioseal F (Ivoclar Vivadent AG) and US (ANOVA, mod LSD $p \geq 0.05$; unpublished data).

The *S mutans*-based artificial mouth model used has proven its reproducibility in producing artificial secondary caries-like lesions during earlier studies.[34-38] To simulate the daily consumption of four cariogenic meals, demineralization was caused by acidogenic *S mutans* producing organic acids during glucose digestion (four, 1-hour demineralization phases, pH Ø 4.2-4.3); remineralization was induced by exposing the specimens to artificial saliva (four, 5-hour remineralization phases, pH Ø 7.0). Nevertheless, it has to be mentioned that using an artificial mouth model with one bacterial strain (in this case *S mutans*, the main pathogenic bacterial species in caries etiology[39-45]) and causing demineralization through acid production during glycolysis is a simplified experimental setup in comparison to the microbiological diversity and complexity of biofilms in the human oral cavity. This simplification constitutes a weakness of the artificial mouth model, and the results should not be unreservedly transferred to the situation *in vivo*. Therefore, the results of *in vitro* studies need to be verified in further *in situ* and *in vivo* studies in order to adhere to an experimental hierarchy with an increasing significance for clinical practice.[20,21]

The fluorescence microscopy evaluation showed that the overall demineralization depths of the FT specimens were significantly smaller at the enamel-pit and fissure sealant interface than those of the other groups (ANOVA, mod LSD, $p<0.05$; Table 4). While a demineralization-inhibiting effectiveness of GICs is verifiable under simulated cariogenic conditions *in vitro,*[34,37] clinical trials show partially contradictory results.[46] It is assumed that an anticariogenic efficacy of fluoride-releasing restoration materials (ie, GICs, compomers) under simulated cariogenic ambient conditions *in vitro* is caused by disrupting bacterial metabolism due to the release of fluoride ions.[46] Fluoride release by the restorative materials depends on material scientific parameters (matrix, fillers, fluoride content, setting reaction) and surrounding factors.[46]

The new, experimental pit and fissure sealant and US did not show significantly different overall demineralization depths in enamel at the pit and fissure sealant margin (ANOVA, mod LSD, $p \geq 0.05$). Therefore, demineralization-inhibiting characteristics of the experimental pit and fissure sealant were not measurable in comparison to US after microbiological loading in the artificial mouth model for 10 days. Under pH-neutral conditions *in vitro*, pit and fissure sealants containing the ion-releasing microcapsules showed a remineralization of initially demineralized artificial carious lesions after 90 days, measured by the fluoride uptake in enamel.[16] The results of the present study showed that under cariogenic conditions in the artificial mouth model, the amount of ion release by the experimental pit and fissure sealant seemed to be insufficient to prevent demineralization.

The surface roughness of glass ionomer cements observed during scanning electron microscopic evaluation has also been described by Yoshihara and others.[47] Changes in the surface quality of sealing materials with increasing roughness and crack formation can encourage biofilm formation,[47] and might be a reason for unfavorable retention rates of GICs used as pit and fissure sealants. According to a meta-analysis by Kühnisch and others, the five-year pooled retention rate estimate amounted to 1.6% for GIC pit and fissure sealants.[48] Because it has an increased surface roughness and shows crack formation, GIC seems best limited to temporary pit and fissure sealing.

Based on the results of the present study, the null hypothesis of no difference in the demineralization-inhibiting effect between the four pit and fissure sealants after microbiological load for 10 days has to be partially rejected and the alternative hypothesis has to be partially accepted, as statistically significant differences among the groups were only measurable at the sealing margins and not at a 500-µm distance from the margin. Additionally, the alternative hypothesis that the four

pit and fissure sealants differ in their surface quality has to be partially accepted, as the appearance of the surface structure showed a material dependence as the appearance of the surface structure was dependent on the type of fissure sealant under investigation.

CONCLUSION

Demineralization-inhibiting effects of the experimental pit and fissure sealant containing microcapsules with remineralizing agents could not be measured after a 10-day exposure to a cariogenic load in a *S mutans*-based artificial mouth model in comparison to a fluoride-releasing resin-based pit and fissure sealant. Within the limitations of this *in vitro* study, the lower demineralization depths at the glass ionomer cement-enamel interface may be caused by anti-cariogenic effects resulting from a burst of fluoride ions released from the mineral fillers[30] during the cariogenic and acidogenic challenge in the artificial mouth model.

Acknowledgments

The authors of this article acknowledge the Premier Dental Company, Plymouth Meeting, PA, USA for supporting this research. Moreover, the authors express their gratitude to the biostatistician Dr Johannes Herrmann for his support of the statistical analysis and to Mrs Lisa Hughes (Premier Dental) for providing information about the sealant composition. Finally, the authors thank Mrs Birgit Meier for performing the EDX analyses.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of Justus-Liebig-University Giessen, Germany. The approval code was AZ 143/09.

Conflict of Interest

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

(Accepted 4 June 2021)

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Interfacial Bond Strength and Morphology of Sound and Caries-affected Dentin Surfaces Bonded to Two Resin-modified Glass Ionomer Cements

RM Al-Hasan • LA Al-Taee

Clinical Relevance

Some resin-modified glass ionomers may enhance outcomes when used with resin composite in stress bearing areas.

SUMMARY

Objective: To evaluate the shear bond strength and interfacial morphology of sound and caries-affected dentin (CAD) bonded to two resin-modified glass ionomer cements (RMGICs) after 24 hours and two months of storage in simulated body fluid at 37°C.

Methods and Materials: Sixty-four permanent human mandibular first molars (32 sound and 32 with occlusal caries, following the International Caries Detection and Assessment System) were selected. Each prepared substrate (sound and CAD) was conditioned (10% polyacrylic acid) and bonded to Activa BioACTIVE Restorative (Activa) and Fuji II LC (F2LC) as per the manufacturers' instructions. Shear bond strength (SBS) was performed after 24 hours and two months of storage. The interfacial surfaces were examined using a digital microscope and scanning electron microscope (SEM). Three-way ANOVA, Bonferroni post-hoc tests (α=0.05), and independent T-tests were used for multifactorial analysis.

Results: Activa exhibited reduced bond strength values to sound and CAD in comparison to F2LC after two time periods (p=0.01). There is a pronounced enhancement in SBS of F2LC when bonded to CAD (p=0.01) after storage, with no statistically significant effect on sound dentin (p=0.309). Activa showed stable SBS to sound and CAD immediately and post-aging (p>0.05).

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http://doi.org/10.2341/21-048-L

However, the evidence of mineral-like deposits under an SEM attached to the aged, debonded dentin surfaces, thereby obliterating the exposed dentinal tubules, might support the tissue repair potential of Activa.

Conclusions: The SBS of Activa was lower than F2LC when bonded to sound and CAD, but the bonding stability and sealing ability is advantageous in minimally invasive therapy, suggesting use with a resin composite coverage when used in stress-bearing areas.

INTRODUCTION

Dental caries is the most predominant bacterially mediated disease worldwide, affecting many people globally and causing considerable health care costs.[1] There are significant challenges in managing deep carious lesions since the traditional complete removal of dental caries compromises pulp health and affects the structural integrity of the tooth. In contrast, incomplete removal of the carious lesion, leaving the deeper layers (caries-affected dentin) that exhibit dentin demineralization but whose collagen framework remains intact without bacterial penetration,[2] will help in arresting caries progression, thereby allowing the remineralization of the residual dentin while maintaining dental pulp vitality.[3] There is a recent focus on using self-adhesive materials with bio-reactive properties that can improve the mineral recovery of tissue. This technique demonstrated satisfactory attributes at the tooth-material interface, producing a high-quality seal that is resistant to contamination from oral fluids, as well as interaction with the microbiological species in addition to restoring the diseased tooth tissues.[4,5]

Glass ionomer cements (GICs) have been used for the Atraumatic Restorative Treatment (ART) techniques as a therapeutic alternative to adhesives/composite restorations. These materials contain a polysalt matrix that exhibits a smart behavior[6] with an ability to release biologically active ions such as fluoride, calcium/strontium, and silicate into the surrounding tissue,[7] thus making them capable of supporting tissue repair and remineralization of the residual caries-affected dentin (CAD) with anti-cariogenic properties.[8] However, the inherent brittleness and solubility of GICs[9] may affect their durability and longevity.[10]

The combination of resin chemistry with the coexistent salt matrix is responsible for the smart interactions of resin-modified glass ionomer cements (RMGICs) with enhanced longevity.[6] They are hybrid materials composed of fluoroaluminosilicate glasses, polyacrylic acid, and resinous materials. The interaction between resin and acid-base reactions may affect the structure and properties of RMGICs, even though they exhibited more enhanced flexural and tensile strengths, elastic modulus, and wear resistance than GICs.[11,12] RMGICs showed an ability to release fluoride and other ions including Na, Ca, Sr, Al, P, and Si as conventional GIC but at different rates.[13] Additionally, RMGICs exhibited higher bonding strength to tooth tissues than conventional GIC, since it is based on both chemical interactions and micromechanical interlocking of the polymer and polyacrylic acid-conditioned tooth surface.[14] The dual setting mechanisms of RMGIC are supposed to encourage relief from polymerization stresses leading to an enhanced marginal seal, despite their lower bond strength compared to resin composites.[15] However, the functionalization of an RMGIC via incorporating a phosphate-based monomer into the liquid phase showed an enhancement in its mechanical and adhesive properties.[16]

ACTIVA BioACTIVE Restorative (Activa) is a functionalized self-adhesive RMGIC with bioactive properties due to the ability to release calcium, phosphate, and fluoride ions.[17] However, Porenczuk and others[18] reported a lower fluoride release profile of Activa compared to Ketac Molar Quick Aplicap but higher than the nanohybrid composite resin Tetric EvoCeram. Previous studies[19,20] reported higher flexural strength, flexural fatigue, and wear resistance of Activa compared to RMGICs and GICs, but they were comparable to that of resin composites. The presence of the hydrophilic ionic resin matrix facilitates the diffusion of ions added to the ability to interact with pH changes, as claimed by the manufacturers, which is supposed to enhance the marginal seal with antimicrobial qualities.[21] However, a one-year randomized clinical study[22] showed a high initial failure rate mainly due to restoration loss and post-operative sensitivity with secondary caries. It is therefore important to investigate the materials' bonding ability and the structural changes of the CAD interface in relevance to the minimal invasive concepts. Therefore, this *in vitro* study compared bonding ability and assessed the interfacial surface changes of sound and CAD surfaces bonded to Activa in comparison to Fuji II LC (F2LC) after 1 day and 60 days of storage in simulated body fluid (SBF) at 37°C. The null hypotheses proposed in this study were: (1) there are no significant differences in the shear bond strength (SBS) of both materials when bonded to sound vs CAD surfaces immediately and post-storage; and (2) there is no significant effect of aging on the SBS of each material per substrate.

METHODS AND MATERIALS

Sixty-four human permanent mandibular first molars (32 sound and 32 with occlusal caries) were collected and stored in distilled water in a cold cabinet (4°C). Samples were hemi-sectioned longitudinally (Isomet 1000, Buehler, Lake Bluff, IL, USA) using a water-cooled diamond blade (330-CA/RS-70300, Struers, LLC, Westlake Cleveland, USA) and embedded in epoxy resin molds. Thirty-two teeth with carious dentin lesions were selected having lesion score 4 following the International Caries Detection and Assessment System (ICDAS),[23] in which the lesion extended through dentin without pulp exposure. The CAD area was inspected visually via detecting the color change[24,25] and by tactile sensation using a dental explorer to examine the consistency and moisture of the different carious zones.[26] Further characterization of the CAD area was carried out using a digital Vickers microhardness machine (TH714, China) by applying 200 gf load for 15 seconds under 400× microscopic magnification. Six measurements (n=2 per zone) were taken in a sequential pattern within each carious lesion, starting from the dentin-enamel junction, by which three zones were identified: caries-infected dentin, CAD, and sound dentin with the Vickers hardness number (VHN) values range for the three zones—17.7-22.7, 27.9-39.0, 43.7-54.5, respectively. All substrates were polished with 600-grit Al_2O_3 abrasive paper under running water using a polishing machine (Laryee Technology CO LTD, China) for 60 seconds, in order to gain flat and smooth surfaces with a standardized smear layer, followed by 3 minutes of ultrasonic cleaning. Then, all surfaces were conditioned using a Dentin Conditioner (10% PAA, GC Corp) for 20 seconds to remove the smear layer, then washed away using air/water spray for 15 seconds and dried for 15 seconds. The experimental groups following the experimental procedures are illustrated in Figure 1.

Shear Bond Strength

Each substrate (sound and CAD, n=32 per group) was subdivided randomly into two groups (n=16), one bonded to Activa and the other to F2LC. Following the manufacturers' instructions (Table 1), the materials were dispensed into cylindrical silicone molds (1.75 mm diameter x 3 mm height) that covered the selected areas completely (Tygon tubing, Saint-Gobain, USA), then photopolymerized using a light-curing device (Model 503, Dentsply, Germany; light intensity of 450 mW/cm^2) for 20 seconds.

A SBS test was performed after 1 day and following 60 days of storage in SBF at 37°C (n=8 per subgroup). The solution was prepared following Kokubo and

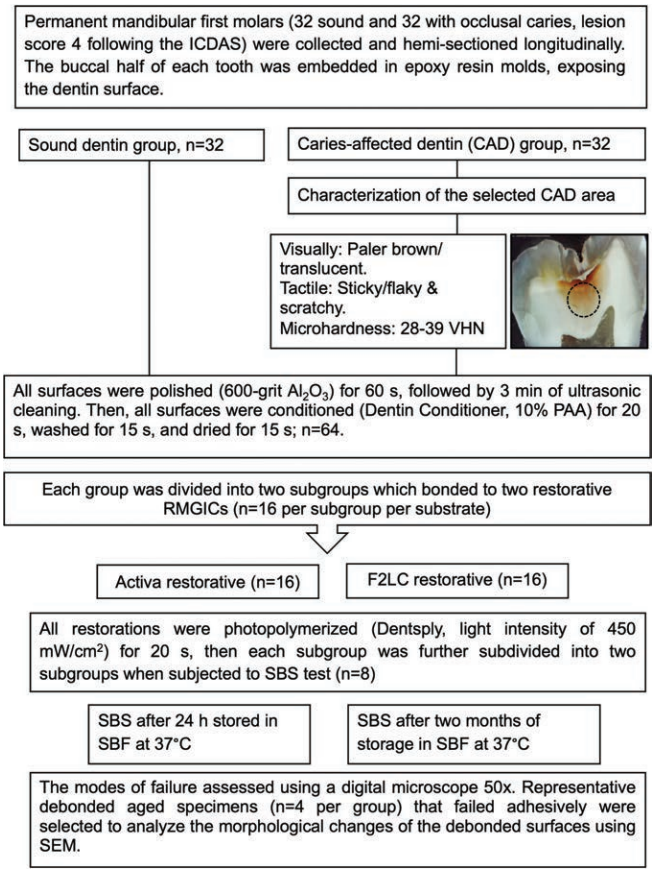

Figure 1. *A scheme representing the experimental groups following the experimental procedures.*

Takadamas' formula[27] to examine the reactivity of Activa for apatite induction and was changed on a weekly basis. The specimens were attached to a universal testing machine (Wdw-50, Laryee Technology Co., China), in which the shear force is directed as close as possible to the tooth/restoration interface. It was applied at a crosshead speed of 0.5 mm/min until failure. The SBS (t) was calculated in MPa using the equation $t=F/(\pi R^2)$ where F was the applied load at failure and R was the radius of the material cylinder.

Mode of Failures and Morphological Assessment of the Debonded Surfaces

The modes of failure were assessed using a digital microscope 50× (Dino-Lite Basic-AM-2011, Taiwan). Then, representative debonded aged specimens (n=4 per group) that failed adhesively were selected to analyze the morphological changes of the debonded surfaces using a scanning electron microscope (SEM; TESCAN, Vega III, Czech Republic) at an accelerating voltage of 10 kV and magnification powers (30×, 500×, and 2500×) and working distances (2 mm, 100 μm, and 20 μm, respectively).

Table 1: *Materials Used and Their Chemical Composition*

Materials and Manufacturers	Composition	Method of Application	Lot No.	Code
Fuji II LC Capsule, Shade A2 GC Corp, Japan	Powder: FAS-glass Liquid: PAA 5%-10%, HEMA 25%-50%, UDMA 1%-5%, Camphorquinone, water.	The capsules were tapped, activated for 10 s and mixed for 10 s (Ultramat 2, 4600 oscillations/min, SDI, Germany). The material was photo-polymerized using a light-curing device (Model 503, Dentsply, Germany), with a light intensity of 450 mW/cm^2 for 20 s.	1909061	F2LC
ACTIVA BioACTIVE Restorative, Shade A2 Pulpdent Corp. USA	Matrix: Diurethane modified by the insertion of a hydrogenated poly-butadiene and other meth-acrylate monomers, modified PAA, sodium, fluoride (0.75%) Filler: 57 wt% (50% bioactive glass & calcium, and 7% silica)	The mixing tip was placed on the Automix syringe. The material was dispensed directly onto the mold and photopolymerized (Model 503, Dentsply, Germany) with a light intensity of 450 mW/cm^2 for 20 s.	190801	Activa
Dentin Conditioner GC Corp, Tokyo, Japan	90% distilled water and 10% polyacrylic acid	Applied for 20 s, washed with air/water spray for 15 s, and dried for 15 s without desiccation	1907101	

Abbreviations: FAS-glass, fluoro-alumino-silicate glass; PAA, polyacrylic acid; HEMA, 2-hydroxyethylmethacrylate; UDMA, urethane dimethacrylate.

Statistical Analysis

The normality tests were performed using Q-Q plots and Shapiro-Wilk. Since the data were normally distributed, further analyses were carried out using three-way analysis-of-variance (ANOVA) and Bonferroni post-hoc tests at the alpha level of significance of 0.05 at each time interval. Then independent t-tests were used to determine the effect of aging on the SBS of each material per substrate, and the differences between substrates ($p<0.05$). The analyses were conducted using SPSS statistical package (version 24, SPSS Inc, IBM, Chicago, IL, USA).

RESULTS

Shear Bond Strength to Sound Dentin

Activa showed statistically significantly lower early and delayed SBS values (3.0±0.5, 2.9±0.4 MPa, respectively) in comparison to F2LC (10.7±2.1, 8.7±2.6 MPa, respectively) ($p<0.01$) when bonded to sound dentin, as shown in Table 2. Aging did not improve the SBS of either material to sound dentin. F2LC showed a lower SBS than its early value, but the difference was statistically insignificant ($p=0.309$), while Activa maintained its bond strength over time ($p=0.943$). The early failures in Activa were predominantly adhesive (87.5%), while in F2LC they were predominantly mixed (50%), combined with 37.5% adhesive and 12.5% cohesive within the cement (Figure 2). However, mixed failures were increased post-aging in F2LC to 75%, and Activa to 50%, combined with less adhesive failures (25% and 50%, respectively).

Shear Bond Strength to Caries-Affected Dentin

Similar to sound substrate, Activa showed reduced SBS values to CAD immediately and post-aging (2.2±0.3, 2.1±0.4 MPa, respectively) compared to F2LC (5.5±1.1, 10.8±2.2 MPa, respectively) ($p=0.01$), as shown in Table 2. The bonding strength of F2LC to CAD was doubled after two months of aging as compared to its early value ($p=0.01$), while it remained constant in Activa post-aging ($p=0.863$). After 24 hours, failures in Activa were entirely adhesive which changed to 75% mixed mode after storage. The same pattern of failures was noticed in F2LC, as they were predominantly adhesive (75%) after 24 hours, but shifted to 25% after storage, as shown in Figure 2.

The difference between substrates (sound vs CAD) had no influence on the bond strength of Activa, immediately and post-storage ($p=0.389$ and 0.337, respectively). In contrast, F2LC showed higher SBS to sound dentin after 24 hours ($p=0.01$) and higher to CAD

Table 2: *Shear Bond Strength (mean MPA±SD) to Sound Dentin and Caries-Affected Dentin After 1 Day and 60 Days Aging in Simulated Body Fluid at 37°C (n=8)*

Materials	Sound Dentin		Caries-Affected Dentin	
	1 d	60 d	1 d	60 d
F2LC	10.7 ± 2.1 ab	8.7 ± 2.6 a	5.5 ± 1.1 a	10.8 ± 2.2 abc
Activa	3.0 ± 0.5	2.9 ± 0.4	2.2 ± 0.3	2.1 ± 0.4

[a]*A statistically significant difference between subgroups in each column (Bonferroni post-hoc tests (α=0.05).*

[b]*A statistically significant difference in values of each material per substrate.*

[c]*A statistically significant effect of aging for each subgroup from day 1 value with in each row (Independent t-test).*

post-aging (p=0.020). The analyzed morphological changes through an SEM of the debonded surfaces are described in Figure 3.

DISCUSSION

The incorporation of energy-absorbing resilient resin matrix in Activa, which is a blend of diurethane and methacrylates with modified polyacrylic acid and polybutadiene modified diurethane dimethacrylate,[28] is supposed to enhance the resilience against impact forces as manifested by high flexural strength and flexural fatigue, which might make it suitable to be used in stress-bearing areas.[19] Additionally, the presence of micro- and nano-hydroxyapatite with a nano-filled RMGIC was reported to reduce the surface roughness changes upon mechanical brushing.[29] On the other hand, the presence of Bioglass in pursuit of enhanced physical properties, combined with the ability to release calcium, phosphate, and fluoride ions, rendered Activa a bioactive restorative material.[17,30] However, the RMGIs already contain reactive, ion-releasing glasses, which in the broadest sense make them bioactive materials. Accordingly, this study was conducted to evaluate whether this class of material is suitable for the Atraumatic Restorative Treatment techniques regarding the bonding efficiency to healthy and diseased tooth tissues, and if there is a potential for tissue repair compared to the widely used RMGIC (F2LC).

Concerning the bonding strength to sound dentin, Activa showed reduced values (3 MPa) immediately and post-aging in comparison to F2LC (<11 MPa), which was even lower than the reported values for RMGICs in the literature (≃7 MPa). [31] The reduced adhesive strength of Activa was also reported in a previous study from Benetti and others[32] who reported a complete loss of all restorations before shear testing when bonded directly to dentin without surface pretreatment. This contradicted the self-adhesion capability that was claimed by the manufacturer, who promised the formation of a resin-hydroxyapatite complex that enhanced the marginal seal against microleakage.[33]

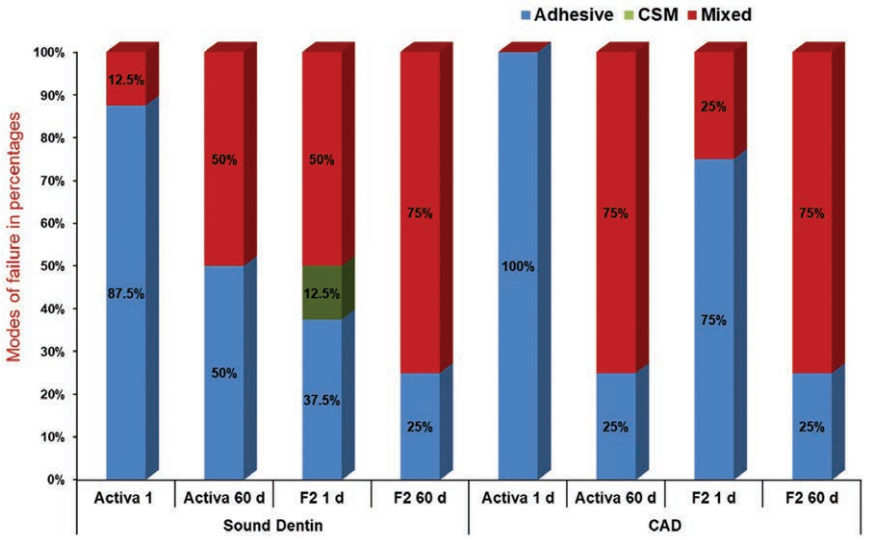

Figure 2. *Modes of failures of Activa and F2LC bonded to sound and caries-affected dentin after 1 day and 60 days of storage in simulated body fluid at 37°C. CSM: cohesive strength within restorative materials.*

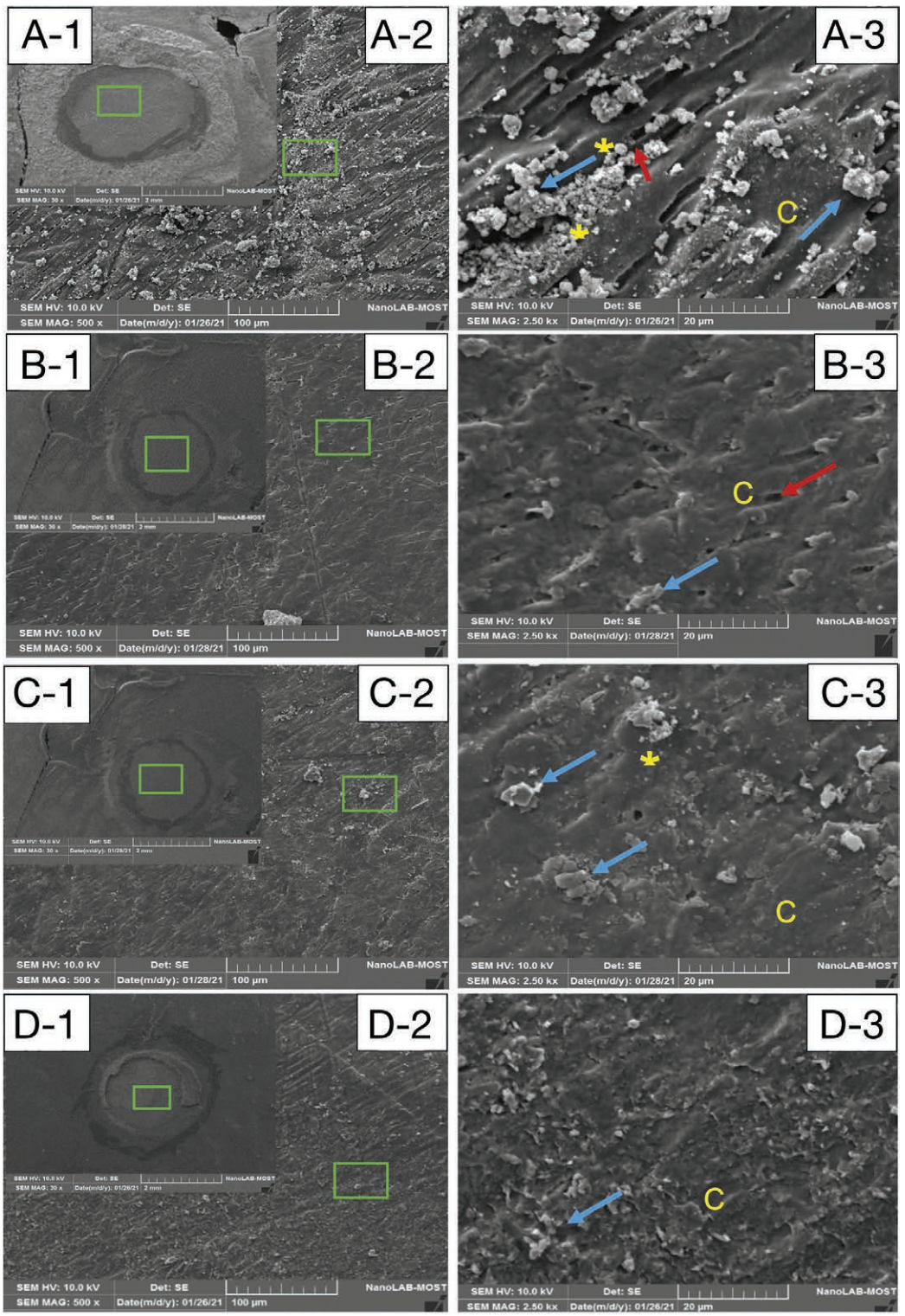

Figure 3. *Representative SEM observations of fractured sound dentin surfaces bonded to Activa (A) and F2LC (B), following two months of storage in simulated body fluid. In A-3 and B-3, failures are cohesive within the hybrid complex showing numerous obliterated dentinal tubules by resinous tags (blue arrows), while some tubules are still open (red arrows), with the remaining cements (C) well integrated to debonded surfaces. In A-3, there is evidence of irregularly shaped granular patches (yellow asterisk) that are attached to the surface and obliterated the dentinal tubules which might indicate the mineral-forming potential of Activa. The SEM observations of the adhesive failures in CAD substrates bonded to Activa and F2LC are shown in C, and D, respectively. The failures occurred within the hybrid layer (C-3, D-3) where the lesions are well sealed by both materials (C), with numerous resinous tags occluding the dentinal tubules (blue arrows).*

Clinical and laboratory studies[21,22,32] reported an enhancement in the bond strength of Activa if they combined with self-etch adhesives, and hence the manufacturers advocated their use recently. However, the use of an adhesive with Activa may interfere with the ion exchange concept, rendering any interfacial bioactive activity very doubtful.[34]

Nevertheless, the bond strength of Activa to dentin was steady after aging accompanied with a shift in the mode of failure from predominantly adhesive (87.5%) to 50% adhesive and mixed patterns, Figure 2, which might indicate the potential chemical integration to sound dentin substrate. This might be related to the reactivity and hydrophilicity of matrix in both cements, which contribute to the acid-base reaction and the formation of stable ionic interactions that endure dissolution and resist the plasticizing effect of water over time[31] and lead to the formation of a homogenous matrix, as evidenced by the SEM images that also showed a dispersion of an irregularly shaped granular mineral kind of deposit covering the aged interface that debonded from Activa associated with completely closed dentinal tubules, as shown in Figure 3 (A-3). This might support the mineral-forming potential of the cement, as claimed by the manufacturer, which would help remineralize the residual carious lesions which requires further investigation. Accordingly, the first hypothesis was rejected, but the second one was accepted as aging does not affect the bond strength of both materials to sound dentin.

The bonding to natural caries dentin is challenging and somewhat unpredictable. This is attributed to the mineral depletion, as presented by a lower Vickers hardness number than sound dentin, the poor quality of hybrid layer, and the extra moisture that might induce hydrolysis of resin and collagen fibrils.[35] These factors might jeopardize the micromechanical interlocking of resin-based polymers producing a lower immediate bond strength in comparison to sound dentin.[36,37] This fact coincides with the finding of this study regarding the reduced initial bond strength of F2LC to CAD than to sound dentin with a higher percentage of adhesive failures (75%) (Figure 2). Although the thick hybrid layer might jeopardize the early micromechanical interlocking into CAD, more ions will be available at the interface for more ionic bonding which might reinforce the carious substrate as presented by a two-fold increase in bond strength after aging, which was higher than that to sound dentin, combined with predominant mixed failures (75%), Figure 2, which might be correlated to the chemical integration of F2LC to CAD surface as shown under an SEM, as the surface appeared well sealed by F2LC with numerous resin tags occluding the exposed tubules (Figure 3, D-3).

The bond strength of Activa to CAD was low (2 MPa), immediately and after storage, compared to F2LC (5.5-10.8 MPa). However, the expected smart behavior of Activa, coupled with the potential chemical bonding due to the ion exchange phenomena,[38] offered a stable bond strength over time without further deterioration. It could be supposed that this ion exchange would be favorable within the residual carious lesion to contribute to the remineralization process, since the lost mineral and apatite crystals can be reintegrated when bonded to bioactive materials,[39] and would potentially protect the restorative-dentin interface from the degradation effect. This was evident through the shift from totally adhesive failures to 75% mixed failures after storage, associated with sealing the carious interface with numerous resinous tags occluding the exposed dentinal tubules under an SEM (Figure 3, C-3). The bond stability of RMGICs to CAD was also revealed in previous studies.[8,40] Accordingly, the first hypothesis was rejected, but the second one was accepted in Activa only, as aging did not affect the bond strength to CAD. Nevertheless, randomized clinical trials are required to justify the use of this promising material regarding tissue repairability and clinical longevity.

CONCLUSIONS

Activa showed reduced bond strength to sound and CAD when compared to F2LC. There was a great enhancement in shear bond strength of F2LC to CAD post storage with no effect of aging noticed in Activa when bonded to sound and CAD. However, the evidence of mineral-like deposits under an SEM attached to the aged debonded dentin surfaces, obliterating the exposed dentinal tubules, might support the tissue repair potential of Activa which might necessitate further investigation.

Acknowledgments

The authors would like to thank the Baghdad College of Dentistry, University of Baghdad, Ministry of Higher Education and Scientific Research, Baghdad/Iraq, for providing the facilities and ultimate support to accomplish this study.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of Baghdad College of Dentistry. The approval code was Reference Number 226.

Conflict of Interest

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

(Accepted 6 Aug 2021)

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The Effect of Different Light-curing Units and Tip Distances on the Polymerization Efficiency of Bulk-fill Materials

HNA Al Nahedh • DF Al-Senan • AS Alayad

Clinical Relevance

Clinicians should exercise caution when selecting and placing resin-based bulk-filling materials using light-curing units for the restoration of deep cavities. Increased distance from the light tip has a detrimental effect on the mechanical properties of composite resin materials.

SUMMARY

Problem Statement: In an average class II posterior preparation, the curing light tip is placed at a distance from the restoration surface that far exceeds the 1-mm manufacturer's recommendation. This distance can have potentially detrimental effects on the curing efficiency of the light-curing unit as well as the properties of the resin-based composite restoration, especially at the bottom of the cavity preparation.

Purpose: The purpose of this study was to evaluate the effects of various types of light-curing units (LCUs) and the different curing distances on the degree of conversion (DC) and the surface hardness of bulk-fill composite materials.

Methods and Materials: A total of 390 specimens of three resin-based composites (RBCs) were fabricated. Two bulk-fill RBCs, including Filtek Bulk Fill Posterior (3M ESPE GmbH, Seefeld, Germany) and Tetric N-Ceram Bulk Fill (Ivoclar Vivadent AG, Schaan, Liechtenstein), as well as a Filtek Z350 XT nano-filled composite (3M ESPE GmbH, Seefeld, Germany), were utilized. In this study, the Vickers microhardness number (VMN) and the DC were evaluated at 2 and 4 mm thicknesses. Polymerization for 20 seconds was performed using two high-power light-curing units, namely the polywave Bluephase G2 light-emitting diode (LED) LCU (Ivoclar Vivadent AG, Schaan, Liechtenstein) and the monowave Elipar Deep Cure S LED LCU (3M Oral Care, St Paul, MN, USA) at 0, 2, and 4 mm distance between the

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http://doi.org/10.2341/20-282-L

curing tip and the RBC surface. The results were analyzed using the two-way analysis of variance method. Scheffe's post-hoc multiple comparison tests were used to determine significant differences between the materials, the LCU, and the tip distances.

Results: The highest DC (70.17) was shown by Filtek Bulk Fill Posterior at a distance of 0 mm, whereas the lowest DC (45.99) was measured for the conventional Filtek Z350 XT at a 4 mm distance. Moreover, higher VMNs were shown by Filtek Bulk Fill and Filtek Z350 composites at 0 mm distance than by the Tetric N-Ceram Bulk Fill composite material when cured with a Bluephase G2 LCU. For all materials, a significant decrease in the DC and mean VMN values was observed at a 4 mm distance in comparison with 0 and 2 mm distances.

Conclusions: The DC and VMN values among the studied bulk fill materials were more significantly affected by the material composition and curing protocols. The increased distance from the light tip has a detrimental effect on the mechanical properties of composite resin materials. Significant differences were observed in the curing efficiency of the two LCUs investigated.

INTRODUCTION

With the development of dental materials, instruments, and clinical techniques, resin-based composites (RBCs) have become the most commonly used materials for direct restoration to satisfy the demands of patients for aesthetics and functional restorative treatment.[1] One of the major problems with RBCs is polymerization shrinkage which generates stress at the tooth restoration interface, resulting in debonding when the bond strength is exceeded by the shrinkage stress.[2] To minimize stress from polymerization shrinkage as well as to acquire adequate mechanical properties for the composite, an incremental placement technique is needed in which the composite is layered and cured in 2-mm increments.[3] However, the technique is quite time-consuming,[4] and if not performed properly, can result in void incorporation in the bulk and at the margins of the restoration, potentially leading to the weakening of the restoration or microleakage.[5] Lately, bulk-fill composites have been developed to simplify the composite resin placement technique. Manufacturers claim that bulk-fill composites create a lower polymerization shrinkage stress and have higher light transmission properties due to a reduction of light scattering at the filler matrix interface by either increasing the scope of the filler or decreasing the quantity of the fillers. Therefore, bulk-fill composites can be used for layers with up to 4-5 mm thickness.[4] Several bulk-fill RBCs are now available, some of which are flowable (low viscosity), whereas some of them are characterized with higher viscosity. High-viscosity bulk-fill RBCs do not require an additional surface layer of conventional hybrid RBC and that they can be used as a single-step bulk-filling material.[6]

Light-curing units (LCUs) play an important role in the development of the basic properties of RBCs. Quartz-tungsten-halogen units have been widely used for the polymerization of RBCs for decades.[7-9] However, they are now largely replaced by light-emitting diode (LED) units. Most of the currently used LED LCUs are second-generational with a single high-powered diode. The improvement of the diode technology allowed an increase in the irradiance of the unit and, accordingly, a decrease in the recommended irradiation time.[10]

There are two main types of LCUs available currently, mono- and polywave LED units. The narrow spectrum of monowave LED LCUs may hinder their ability to optimally cure bulk-fill composites with multiple photoinitiators with varying peak absorption ranges. However, polywave LED LCUs (third-generational) can radiate different wavelengths of light to polymerize different photoinitiators.[11]

During the curing process, some of the light is reflected off the surface of the RBC, and some light that passes through the RBC is absorbed or scattered based on the particle size of the fillers as well as the refractive indices of the resin matrix and the fillers. Consequently, the intensity of the light is decreased and its effectiveness is reduced as the depth increases.[12] Meanwhile, the composition as well as the initiator systems of the bulk-fills are comparable to those of the conventional RBCs.[13]

Light intensity diminishes when the distance from the tip of the light source to the resin composite is increased. Therefore, the most common clinical recommendation for the position of the tip is 1 mm from the resin.[14]

A previous study evaluated the impact of the distance between the light guide tip of the curing unit and material surface on the DC and Knoop microhardness of a composite resin. Their results showed that increased curing distance can affect the mechanical properties of composite restoration.[15] Another study has also shown that greater tip distances produce a decrease in microhardness and DC values.[16] However, a similar correlation was not performed for bulk-fill RBCs.

The purpose of the present study was to evaluate the effects of different LCU types as well as the distance from the LCU tip on the DC and the surface microhardness of bulk-fill composite materials.

The null hypotheses of this study were that there would be no significant differences in the DC of two bulk-fill composites after polymerization with different LCUs, no differences in using different distances between the LCU tips and the restoration surface on curing parameters and surface hardness, and no differences in surface hardness with the application of different LCUs.

METHODS AND MATERIALS

Three-hundred and ninety specimens of two bulk-fill resin composites (Filtek Bulk Fill Posterior, shade A2 [3M ESPE; St Paul, MN], Tetric N-Ceram Bulk Fill [universal A shade; Ivoclar Vivadent; Schaan, Liechtenstein], and Filtek Z350 XT conventional nano-filled composite resin [shade A2; 3M ESPE]) were used in this study (Table 1). The microhardness and the DCs were evaluated at 2- and 4-mm thicknesses after polymerization using two LCUs, including polywave Bluephase G2 LED LCU (Ivoclar Vivadent) and monowave LED LCU (3M ESPE). The light-curing tip was positioned at distances of 0, 2, and 4 mm from the surface of the composite material. The curing time was 20 seconds for the two LCUs.

Specimen Preparation

Disk-shaped specimens were fabricated from the two bulk-fill materials to be used in hardness measurements (n=120). A special custom sectional Teflon mold (10 mm in diameter and 4 mm deep) was used, the uncured paste of each composite was placed in two layers, each of which was 2 mm thick and the layers were separated by a celluloid strip. Sixty specimens for each material were fabricated using either Bluephase G2LCU or Elipar DeepCure-S (n=30) at 0, 2, and 4 mm distance (n=10). The distance from the composite surface was calibrated and stabilized using a laboratory ring and clamp stand (Dentalfarm; Torino, Italy). Vickers microhardness was measured on both sides of each layer of these discs.

For evaluation of the degree of conversion, rectangular specimens (n=120) were fabricated from the two composite materials (n=60) using a custom Teflon mold (6 mm in length, 3 mm in width, and 4 mm in depth). The materials were placed in the mold over a glass slab. After insertion into the mold, a glass plate of 1.00 mm thickness was secured over the mold to

Table 1: *Resin Composite Materials Used in the Study*

Materials/Shade	Lot Number	Material Type	Resin Matrix	Filler
Tetric N-Ceram Bulk Fill (Ivoclar-Vivadent, Liechtenstein) Shade IVB The European trade name Tetric EvoCeram Bulk-Fill	T47219	Packable hybrid bulk-fill composite	Bis-GMA, Bis-EMA, and UDMA	Barium glass, prepolymer, ytterbium trifluoride, and mixed oxide. Inorganic filler particle size is between 0.04 μm and 3 μm, mean particle size is 0.6 μm Filler loading 75-77% by wt, 53-55% by volume.
Filtek Bulk Fill Posterior Restorative (3M ESPE, USA) Shade A2	N682081	Packable nanofilled bulk-fill composite	ERGP-DMA, diurethane-DMA, and 1, 12-dodecane-DMA	Non-agglomerated/non-aggregated 20-nm silica filler, 4-11-nm zirconia filler, aggregated zirconia/silica cluster filler, and ytterbium trifluoride filler agglomerate 100 nm particles. Filler loading 76.5% by wt, 58.4% by volume
Filtek Z350 XT (3M ESPE, USA) A2 Body shade Trade name in North America Filtek Supreme Ultra	N677462	Nanohybrid composite	Bis-GMA, UDMA, TEGDMA, PEGDMA, and Bis-EMA	Non-agglomerated/non-aggregated 20-nm silica filler, 4-11-nm zirconia filler, and aggregated zirconia/silica cluster filler. Filler loading 78.5% by wt, 63.3% by volume

Abbreviations: UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; Bis-GMA, bisphenol A-glycidyl methacrylate; Bis-EMA, ethoxylated bisphenol-A dimethacrylate; ERGP-DMA, ERGP- dimethacrylate; PEGDMA, Polyethylene glycol dimethacrylate.

flatten the surface. Specimens were divided according to the type of applied LCU into 2 groups (n=30) and afterward subdivided according to tip distances into 3 groups (n=10). The LCU tip was placed at a 0, 2, and 4 mm distance from the top surface of the specimen and subsequently cured for 20 seconds. For each tip distance group, DC measurements were performed (n=10).

Conventional Filtek Z350 XT (3M ESPE) was used as control. Disk-shaped specimens were fabricated to be used in hardness measurements (n=60) using a Teflon mold (10 mm in diameter and 2-mm deep), the specimens were divided into two groups according to the type of LCU (n=30) and subsequently subdivided based on the distances from the LCU tip into three groups (n=10). The DC specimens (n=60) were 6 mm long, 3 mm wide, and 2 mm deep and were cured for 20 seconds at 0, 2, and 4 mm distances. For each LCU and distance subgroup, 10 specimens were fabricated.

Thirty uncured specimens of the three composite materials were placed over the ATR crystal (n=10), and the spectrum of the uncured material was recorded for the duration of one scan.

The analysis and measurement of the irradiance values, spectral emission, and radiant exposure delivered to each specimen at 0, 2, and 4 mm distances were performed using a MARC-RC device (BlueLight Analytics; Halifax, Canada), as shown in Figure 1.

Microhardness Measurement (VMN)

Microhardness was measured using a Vickers hardness tester immediately after the fabrication of the specimen (InnovaTest Europe BV; Maastricht, the Netherlands). The surface of each specimen was subsequently divided into thirds. Three indentations were introduced, one in the center of each third using a Vickers microhardness indenter with 300g load applied for 15 seconds. Measurements were performed on the top surface of the top layer, the bottom surface of the top layer, the top surface of the bottom layer, and the bottom surface of the bottom layer. Afterward, the mean microhardness (VMN) values were calculated for each surface.

Bottom-to-top surface hardness ratios were calculated separately for the top and bottom layers and the full thickness (bottom surface at 4 mm depth/top surface hardness).

Degree of Conversion Measurement

The DC measurements were performed from the uncured material immediately after removal from the syringe, and the irradiated specimen surface within 2 hours of curing. After photoactivation, absorbance peaks were obtained through the transmission mode of a Fourier-transform infrared spectrometer (FTIR, Nicolet iS10 Series; Thermo Scientific, Waltham, MA). The excitation was an Nd:YAG (neodymium-doped yttrium aluminum garnet) laser at 1038 nm with a laser power of 800 mW and a resolution of 4 cm^{-1}. The spectra of the uncured composites were recorded in the same manner.

DC calculations were performed by comparing the relative change of the band at 1638 cm^{-1} representing the aliphatic C=C stretching mode to the aromatic C=C band at 1608 cm^{-1}, before and after polymerization. The integrated intensities of the aliphatic and aromatic C=C bands were used for the DC calculation based on the following equation:

$$DC\ (\%) = 1 - R_{polymerized}/R_{unpolymerized},$$

where R = (aliphatic C=C band area)/(aromatic C=C band area).

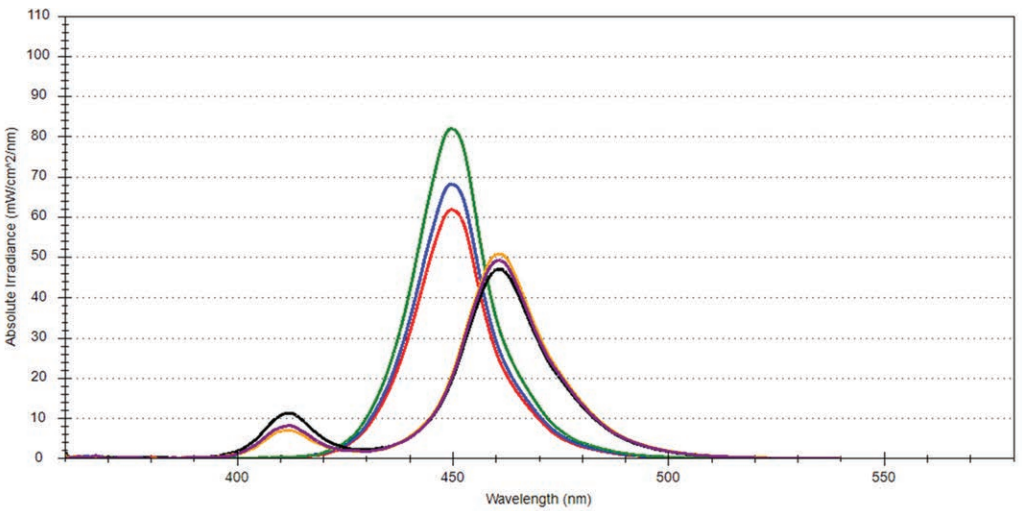

Figure 1. *Spectrum emission for Blue Phase G2 and Deep Cure S at 0, 2, and 4 mm distances for 20 seconds. The short lines on the right refer to Blue Phase G2 and the longer lines on the left refer to Deep Cure S.*

Statistical Analyses

Data were statistically analyzed using SPSS (Statistical Package for the Social Sciences) version 22 for Windows (IBM, Armonk, NY) and the Shapiro–Wilk test was applied for normality testing. Moreover, the homogeneity of variance was analyzed by the Levene's test. Data were presented as means and standard deviation (SD). Due to the detected heterogeneity of variance between the different groups of composites, the two-way analysis of variance (ANOVA) method followed by the one-way ANOVA was used. Scheffe's post-hoc multiple comparison tests were used for the determination of significant differences between the materials, LCUs, and tip distances. Furthermore, multiple regression analyses for the DCs were also carried out at the three locations (top, middle, and bottom). The results were analyzed assuming a significance level of 0.05, at which the statistical power was satisfactory (80%) for the detection of medium-size effects (Cohen's f=0.25).

RESULTS

Microhardness (VMN)

The two-way ANOVA results confirmed that the material, LCU type, and different light tip distances had significant effects on the mean VMN results ($p<0.05$). However, the interaction between the material and the different light tip distances as well as between the curing type and the different light tip distances had no significant effect on the mean VMN results ($p>0.05$).

Top Layer—The bulk-fill materials differed significantly in their VMN ratios (Table 2). The highest microhardness at 0 mm and mean MH ratio (0.984±0.005) were shown by the Filtek Bulk Fill Posterior when cured with the Bluephase G2 LCU, while the lowest mean MH at 4 mm was shown by the Tetric N-Ceram Bulk Fill with DeepCure-S LCU (0.921±0.002). Moreover, the Filtek Bulk Fill Posterior was observed to have higher microhardness values in comparison with the Tetric N-Ceram Bulk Fill at all distances except when cured with the Bluephase G2 LCU at 4 mm (Figure 2). Both materials showed lower microhardness ratios when cured with the DeepCure-S LCU in comparison with the Bluephase G2 LCU.

Significant differences were observed between the three tip distances with all material LCU combinations except for the Tetric N-Ceram Bulk Fill cured with the Bluephase G2 where no significant differences were found between 2 and 4 mm LCU distances.

Bottom Layer—The highest mean VMN (0.837±0.003) was shown by the Filtek Bulk Fill Posterior composite (DeepCure-S, 0 mm), whereas the lowest mean VMN (0.608±0.005) was measured in association with the Tetric N-Ceram Bulk Fill (DeepCure-S, 4 mm).

The Filtek Bulk Fill Posterior composite cured with the Bluephase G2 LCU at 0 mm showed a significantly higher microhardness in comparison with the Tetric N-Ceram Bulk Fill composite material. Furthermore, Tetric N-Ceram Bulk Fill cured with the DeepCure-S

Table 2: *Results of Two-way ANOVA Showing Mean (± SD) VMN Ratios for the Top Layer and Significant Differences Between the Three Distances for Each Material and LCU Combination*

Material	Curing Type	Light Tip Distance (mm)	Mean[a]	Standard Deviation	Df	Mean Square	F	Sig
Tetric N-Ceram Bulk Fill	Blue Phase G2	0	0.980 a	0.004	2	0.001	65.90	0.000
		2	0.967 b	0.002				
		4	0.964 b	0.003				
	Deep Cure S	0	0.941 a	0.004	2	0.001	60.95	0.000
		2	0.930 b	0.006				
		4	0.920 c	0.002				
Filtek Bulk Fill	Blue Phase G2	0	0.984 a	0.005	2	0.006	56.03	0.000
		2	0.969 b	0.002				
		4	0.936 c	0.017				
	Deep Cure S	0	0.97 2a	0.002	2	0.008	862.86	0.000
		2	0.968 b	0.002				
		4	0.921 c	0.004				

[a] *Lowercase letters show the differences within distances for each material and light-curing unit.*

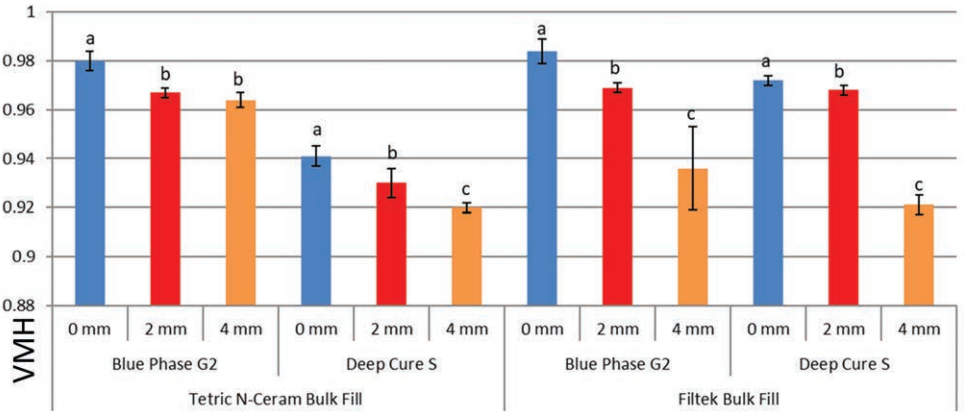

Figure 2. *Bar chart of mean (± SD) microhardness (VMH) ratios for the top surface. Lowercase superscript letters show differences within distances for each material and light curing unit.*

LCU at 4 mm displayed the lowest microhardness ratio. Moreover, microhardness values were found to be higher for both bulk-fill materials when cured with the Bluephase G2 LCU compared to the DeepCure-S LCU (Figure 3). There were significant differences among all three LCU tip distances for Tetric N-Ceram cured with Bluephase G2 and DeepCure-S, however, the Filtek Bulk Fill Posterior showed significant difference with DeepCure-S but not with Bluephase G2 (Table 3).

Full Thickness— The highest mean VMN (0.969±0.008) was attributed to the Filtek Z350 (Bluephase G2, 0 mm), whereas the lowest mean VMN (0.618±0.005) was observed in association with the Tetric N-Ceram Bulk Fill (Bluephase G2, 4 mm) (Figure 4, Table 4). In addition, there was no significant difference measured for the ratio of top to bottom surface microhardness values among the Filtek Bulk Fill and the Filtek Z350 nanohybrid composites at 0 mm distance when cured with Bluephase G2 LCU. Both of them were found to have significantly higher microhardness ratios than the Tetric N-Ceram Bulk Fill composite material, which showed lower microhardness values at 4 mm distance when cured with Bluephase G2 LCU than the former two composites. Besides, all distances revealed significant differences except for the Filtek Bulk Fill Posterior when cured with Bluephase G2.

Multiple regression was performed to predict MH for the material, the curing type, and the distance of the light cure tip. These variables predicted the MH with statistical significance, $F(3,146) = 177.753$, $p < 0.0005$, $R2 = 0.785$. All three variables added significantly to the prediction, $p < 0.0005$ (Table 5).

Degree of Conversion (DC)

Results of the two-way ANOVA showed that the material, LCU type, light tip distance, and the interaction between the three variables for all three locations (top, middle, and bottom) had significant effects on the mean DC ($p<0.001$) (Table 6). The results of the Scheffe's post-hoc tests of the mean differences in the DC between the variables were significant (Table 7). In addition, all materials showed a higher DC when cured at 0 mm distance in comparison with that at 2 and 4 mm as well as a significantly higher DC at the

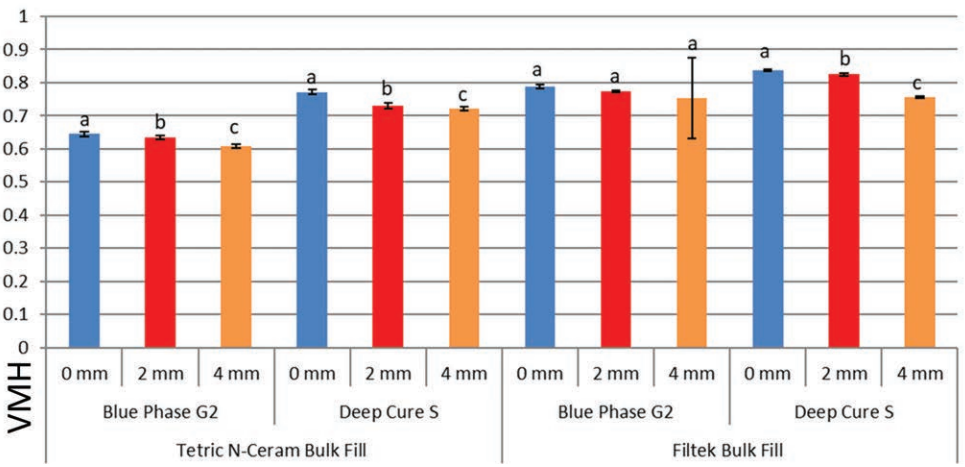

Figure 3. *Bar chart of mean (± SD) microhardness (VMH) ratios for the bottom surface. Lowercase superscript letters show differences within distances for each material and light curing unit.*

Table 3: *Results of Two-way ANOVA Showing Mean (± SD) VMN Ratios for the Bottom Layer and the Significant Differences Between the Three Distances for Each Material and LCU Combination*

Material	Curing Type	Light Tip Distance (mm)	Mean[a]	Standard Deviation	Df	Mean Square	F	Sig
Tetric N-Ceram Bulk Fill	Blue Phase G2	0	0.645 a	0.008	2	0.004	97.93	0.000
		2	0.634 b	0.005				
		4	0.608 c	0.005				
	Deep Cure S	0	0.772 a	0.006	2	0.007	150.22	0.000
		2	0.731 b	0.009				
		4	0.721 c	0.005				
Filtek Bulk Fill	Blue Phase G2	0	0.787 a	0.006	2	0.003	.539	0.589
		2	0.773 a	0.003				
		4	0.754 a	0.122				
	Deep Cure S	0	0.837 a	0.003	2	0.019	2158.54	0.000
		2	0.825 b	0.004				
		4	0.756 c	0.002				

[a] *Lowercase letters show the differences within distances for each material and light-curing unit.*

top surface than the middle or bottom surfaces. The examination of the percentage reduction in the DC values across the different LC tip distances and within each distance indicated that the worst performance was in connection with the DeepCure-S at 4 mm LC tip distance (Table 6).

Top—The highest mean DC (70.17±0.37) was measured with the Filtek Bulk Fill Posterior (Bluephase G2, 0 mm), whereas the lowest (45.99±0.46) was observed with the Filtek Z350 (DeepCure-S, 4 mm) (Figure 5).

Middle (2-mm Thickness)—The highest mean DC (68.90±0.450) was found with the application of the Filtek Bulk Fill Posterior (Bluephase G2, 0 mm), whereas the least (47.54±0.168) was obtained with the Tetric N-Ceram Bulk Fill (DeepCure-S, 4 mm) (Figure 6).

The Filtek Bulk Fill Posterior cured at 0 mm showed a significantly higher DC than both the Tetric N-Ceram and the Filtek Z350 XT when cured with the Bluephase G2 LCU. Also, the Tetric N-Ceram Bulk Fill and the Filtek Z350 XT revealed significantly higher DCs at 0 mm in comparison with those at 2 and 4 mm. All three materials showed significantly higher DCs when cured with the Bluephase G2 LCU compared to the DeepCure-S LCU ($p<0.05$).

Bottom—The highest mean DC (65.56±0.21) was found with the Filtek Bulk Fill Posterior (Bluephase G2,

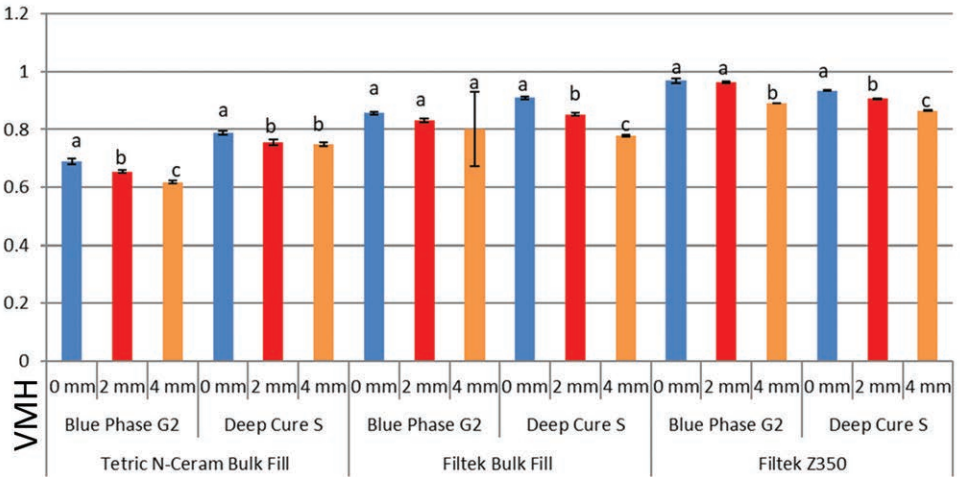

Figure 4. *Bar chart of mean (± SD) microhardness (VMH) ratios for full (4 mm) thickness. Lowercase superscript letters show differences within distances for each material and light curing unit.*

Table 4: *Results of Two-way ANOVA Showing Mean (± SD) VMN Ratios for the Full Thickness and Significant Differences Between the Three Distances for Each Material and LCU Combination*

Material	Curing Type	Light Tip Distance (mm)	Mean[a]	Standard Deviation	Df	Mean Square	F	Sig
Tetric N-Ceram Bulk Fill	Blue phase G2	0	0.689 a	0.011	2	0.013	235.75	0.000
		2	0.654 b	0.005				
		4	0.618 c	0.005				
	Deep Cure S	0	0.787 a	0.007	2	0.004	61.66	0.000
		2	0.756 b	0.010				
		4	0.748 b	0.007				
Filtek Bulk Fill	Blue phase G2	0	0.856 a	0.006	2	0.008	1.33	0.280
		2	0.831 a	0.008				
		4	0.801 a	0.130				
	Deep Cure S	0	0.909 a	0.005	2	0.043	3113.96	0.000
		2	0.852 b	0.004				
		4	0.778 c	0.002				
Filtek Z350	Blue phase G2	0	0.969 a	0.008	2	0.009	343.61	0.000
		2	0.962 a	0.003				
		4	0.891 b	0.000				
	Deep Cure S	0	0.934 a	0.002	2	0 .006	1380.30	0.000
		2	0.906 b	0.001				
		4	0.865 c	0.002				

[a] *Lowercase letters show the differences within distances for each material and light-curing unit.*

0 mm), whereas the lowest (29.96±0.51) was displayed with the Tetric N-Ceram Bulk Fill (DeepCure-S, 4 mm) (Figure 7).

The one-way ANOVA analysis showed significant differences between the LCU types (p<0.05) for all locations (Table 8). Multiple regression was run to predict the DC (all locations) for the material, curing type, and distance of the light cure tip. The DC was predicted with statistical significance by these variables, p < 0.0005. All three variables added significantly to the prediction, p < 0.05 for all locations except for the bottom where only two variables (the LCU type and the LC tip distance) added to the prediction. Moreover, there was a statistically significant difference identified between the materials, curing type, and tip distances (p<0.05). The DC showed a significant reduction with increasing distance of the LCU from the composite resin surface (Table 9).

Table 5: *Multiple Regressions for Full-Thickness VMN Values*

Coefficients							
Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	0.615	0.018		33.89	0.000	0.580	0.651
Material	0.109	0.005	0.807	21.04	0.000	0.099	0.120
LCU	0.043	0.008	0.212	5.52	0.000	0.028	0.058
Distance	-0.037	0.005	-0.297	-7.75	0.000	-0.046	-0.028
F (3,146)=177.753			R2=0.785			p<0.0005	

Table 6: *Result of Two-way ANOVA Showing Mean (±SD) of DC Values and Significant Differences Between the Three Distances for Each Material and LCU for All Three Locations (Top, Middle, and Bottom) Combination*

Material LCU	Tip mm	Top Mean (SD)[a]	2 mm Mean (SD)[a]	Bottom Mean (SD)[a]	% DC Reduction LC Distance		Bottom / Top
					Top	Bottom	Full
Tetric N-Ceram Blue phase G2	0	68.95 a (.409)	67.64 a (.194)	64.51 a (.936)	95 (2/0)	89 (2/0)	94 (0)
	2	65.17 b (.493)	63.21 b (.387)	57.48 b (.133)	77 (4/2)	61(4/2)	88 (2)
	4	50.39 c (.144)	49.51 c (.143)	35.30 c (.408)	73 (4/0)	55 (4/0)	70 (4)
Tetric N-Ceram Deep Cure S	0	66.64 a (.150)	65.98 a (.557)	63.97 a (.733)	93 (2/0)	85 (2/0)	96 (0)
	2	61.74 b (.477)	60.49 b (.130)	54.58 b (.746)	80 (4/2)	55 (4/2)	88 (2)
	4	49.30 c (.520)	47.54 c (.168)	29.96 c (.511)	74 (4/0)	47 (4/0)	61 (4)
Filtek Bulk Fill Blue phase G2	0	70.17 a (.373)	68.90 a (.497)	65.56 a (.211)	95 (2/0)	92 (2/0)	93 (0)
	2	66.54 b (.148)	65.50 b (.099)	60.53 b (.111)	79 (4/2)	62 (4/2)	91 (2)
	4	52.47 c (.074)	50.41 c (.089)	37.66 c (.366)	75 (4/0)	57 (4/0)	72 (4)
Filtek Bulk Fill Deep Cure S	0	67.62 a (.118)	65.60 a (.147)	64.19 a (.531)	95 (2/0)	88 (2/0)	95 (0)
	2	64.33 b (.287)	62.44 b (.130)	56.37 b (.297)	78 (4/2)	61 (4/2)	88 (2)
	4	50.31 c (.120)	48.44 c (.089)	34.45 c (.164)	74 (4/0)	54 (4/0)	68 (4)
Filtek Z350 Blue phase G2	0	65.42 a (.131)		63.36[a] (.091)	96 (2/0)	92 (2/0)	97 (0)
	2	62.48 b (.097)		58.52[b] (.126)	84 (4/2)	61 (4/2)	94 (2)
	4	52.38 c (.096)		35.53[c] (.140)	80 (4/0)	56 (4/0)	68 (4)
Filtek Z350 Deep Cure S	0	62.41 a (.087)		59.61[a] (.092)	94 (2/0)	92 (2/0)	96 (0)
	2	58.57 b (.130)		54.57[b] (.117)	79 (4/2)	56 (4/2)	93 (2)
	4	45.99 c (.462)		30.32[c] (.448)	74 (4/0)	51 (4/0)	66 (4)

[a]*Lowercase letters show the differences within distances for each material as well as the light-curing unit ($p<0.001$) for all locations.*

DISCUSSION

In the present study, the effects of different LCUs and tip distances were tested on the polymerization efficiency of bulk-fill composites. The results of this study showed that all tested RBC materials presented higher DCs when cured with the Bluephase G2 LCU compared to the DeepCure-S LCU. Therefore, the first null hypothesis was rejected. Polywave LED LCUs are used to activate a wider range of photoinitiators, some of which require shorter wavelengths of light, and due to that narrow-spectrum LED LCUs emit very little light below 420 nm, single-peak LED lights are not very effective and might produce weaker RBC restorations.[17] This is in agreement with the results reported by Price and others,[18] who compared the effects of second- and third-generational LED LCUs on the microhardness of various RBCs.

When cured with the Bluephase G2 LCU, the Filtek Bulk Fill Posterior indicated a significantly higher DC at the top and the bottom layers than the Tetric N-Ceram Bulk Fill and the Filtek Z350 XT RBC materials. However, there were no significant differences observed at the 2 mm depth. These results are quite surprising considering that the Bluephase G2 is a polywave unit with an ultraviolet light spectrum (– 410 nm) which excites the Ivocerin (bis (4-methoxybenzoyl) diethyl-germane Ge-3) initiator that is present in the Tetric N-Ceram. However, the monowave DeepCure-S LCU has a higher light irradiance than the Bluephase G2 with a wavelength covering the 440-500 nm range, that is the excitation peak of Ivocerin (408-440 nm). Also, previous studies reported limited penetration of the ultraviolet wavelengths into the composite as they get depleted in its top layer.[11] Therefore, it is likely that other factors related to the LCU or the materials also influence the results. The presence of multiple chips in the head of the Bluephase G2 LCU may lead to more even light distribution at the surface and throughout the composite.

Shimokawa and others reported that the total amount of light reaching the bottom of the 4-mm-thick specimens was only about 10% of the light delivered to the top, and

Table 7: *Scheffe Post-Hoc Comparison of the Mean Difference in DC Between the Different Materials, Light Cure Units, and Tip Distances for All Three Locations (Top, Middle, and Bottom)*

Dependent Variables	Location	Mean Difference			Std Error	Sig	Significance Between LC Tip Distances
		0-2	2-4	0-4			
Tetric N Ceram X Blue Phase G2	Top	3.78	14.77	18.56	0.169	<0.001	0 mm > 2 mm > 4 mm
	Middle	4.42	13.69	18.12	0.117	<0.001	
	Bottom	7.03	22.17	29.20	0.266	<0.001	
Tetric N Ceram X Deep Cure S	Top	4.89	12.44	17.34	0.169	<0.001	
	Middle	5.49	12.94	18.44	0.154	<0.001	
	Bottom	9.38	24.62	34.00	0.300	<0.001	
Filtek Bulk Fill X Blue Phase G2	Top	3.63	14.07	17.70	0.105	<0.001	0 mm > 2 mm > 4 mm
	Middle	3.39	15.08	18.48	0.132	<0.001	
	Bottom	5.02	22.87	27.90	0.112	<0.001	
Filtek Bulk Fill X Deep Cure S	Top	3.28	14.01	17.30	0.086	<0.001	
	Middle	3.15	14.00	17.15	0.055	<0.001	
	Bottom	7.81	21.92	29.74	0.162	<0.001	
Filtek Z350 X Blue Phase G2	Top	2.94	10.09	13.03	0.069	<0.001	0 mm > 2 mm > 4 mm
	Bottom	4.84	22.99	27.83	0.076	<0.001	
Filtek Z350 X Deep Cure S	Top	3.83	12.58	16.41	0.178	<0.001	
	Bottom	5.03	24.24	29.28	0.172	<0.001	

the spectral radiant power ratio of the delivered violet to blue light dropped from 26% (top) to only 2% at the bottom of the RBC specimen. This occurs as the light is used, absorbed, or reflected by the specimen during the polymerization reaction. However, the reduced amount of light as well as the limited penetration of the violet wavelengths may lead to inadequate polymerization in the deepest regions of the restorations, especially for the Tetric N-Ceram Bulk Fill.[19]

The DC of the top surface decreases significantly with an increased LCU tip distance. For all materials, a significant drop in the DC was quite evident at the 4 mm distance in comparison with the 0 and 2 mm distances. Therefore, the second null hypothesis was rejected. Moreover, the Tetric N-Ceram Bulk Fill had a lower DC than the Filtek Bulk Fill Posterior, which was in agreement with a previous study.[20] This difference in the DC results is probably due to a varying matrix and filler content of the two materials. Filtek Bulk Fill Posterior was reported to have uniformly small filler particles (1-3 μm), whereas the Tetric N-Ceram Bulk Fill was demonstrated to contain a wide range of particle sizes (1-30 μm) of prepolymerized resin particles and aggregates that were previously filled with fused silica, which could possibly affect its mechanical properties.[21] Regarding the microhardness at 4 mm, all ratios for the Tetric N-Ceram Bulk Fill cured by either LCU were below the acceptable level of 0.8 (or 80%). As

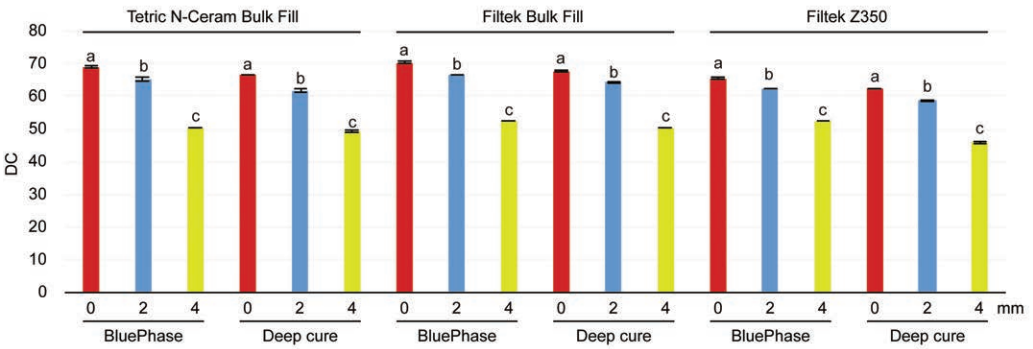

Figure 5. *Bar chart of mean (± SD) degree of conversion (DC) values (top 0 mm). Lowercase superscript letters show differences within distances for each material and light curing unit.*

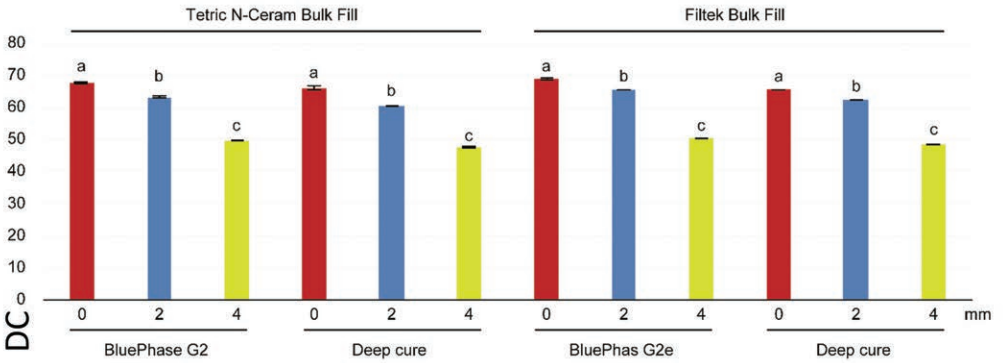

Figure 6. *Bar chart of mean (± SD) degree of conversion (DC) values (middle 2 mm). Lowercase superscript letters show differences within distances for each material and light curing unit.*

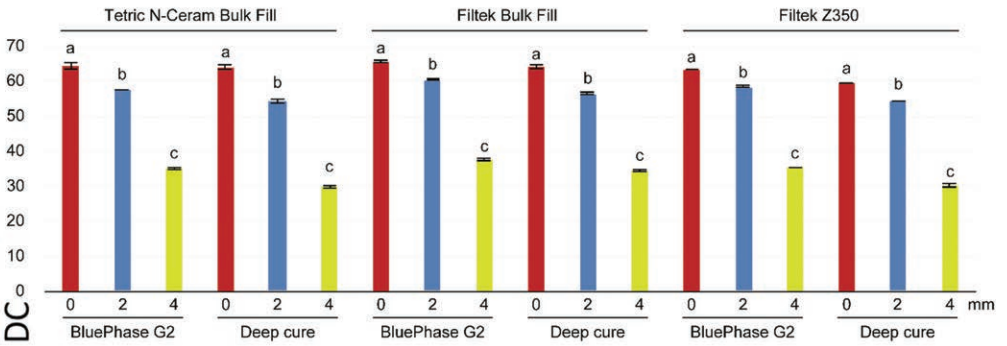

Figure 7. *Bar chart of mean (± SD) degree of conversion (DC) values (bottom 4 mm). Lowercase superscript letters show differences within distances for each material and light curing unit.*

for the Filtek Bulk Fill Posterior, all values were above 0.8 except for the 4 mm distance with the DeepCure-S. This is a direct result of the higher DC displayed by this material.

Similar to the DC results, the microhardness values for the Filtek Bulk Fill Posterior were higher than those of the Tetric N-Ceram Bulk Fill when cured with the Bluephase G2 LCU. Generally, all materials exhibited significantly lower microhardness values when cured with the DeepCure-S LCU, with the Tetric N-Ceram Bulk Fill revealing the lowest bottom-to-top surface VMN ratios. Therefore, the third null hypothesis was rejected. The spectrum emission for the Bluephase G2 and the Deep Cure S at 0, 2, and 4 mm distances for 20 seconds showed that the DeepCure-S LCU had a significant drop in the absolute irradiance from 82 to 62 mW/cm^2/nm while the Bluephase G2 presented a minor drop, which might explain the less-than-optimal performance of this LCU at greater distances.

According to Price and others,[18] several different types of LED chips are used by third-generation LED LCUs for the delivery of a broader spectral output in comparison with the narrower spectral output of second-generation LCUs, which can result in better mechanical properties of the RBCs. Another possible explanation is that the DeepCure-S has a collimated beam and higher irradiance and can, therefore, still reach photoinitiators at greater depths.[22]

Table 8: *One-way ANOVA Showing a Statistically Significant Difference Between Cure Types (p<0.05) for All Locations*

LCU	Location	Mean	Std Error	95% Confidence Interval		Sig *p*<0.05
				Lower Bound	Upper Bound	
Blue Phase G2	Top	61.556	0.037	61.483	61.629	0.00
Deep Cure S		58.551	0.037	58.478	58.625	0.00
Blue Phase G2	Middle	60.86	0.035	60.79	60.93	0.00
Deep Cure S		58.41	0.035	58.34	58.48	0.00
Blue Phase G2	Bottom	53.16	0.056	53.05	53.27	0.00
Deep Cure S		49.78	0.056	49.67	49.89	0.00

Table 9: *Multiple Regression Results for DC (Top Surface, 2 mm, and Bottom)*

Coefficients								
Model		**Unstandardized Coefficients**		**Standardized Coefficients**			**95% Confidence Interval for B**	
		B	**Std Error**	**Beta**	**t**	**Sig**	**Lower Bound**	**Upper Bound**
Top	Material	-0.848	0.304	-0.083	-2.78	0.006	-1.45	-0.246
	LCU	-2.71	0.456	-0.177	-5.96	0	-3.619	-1.818
	Distance	-8.56	0.279	-0.912	-30.69	0	-9.115	-8.012
	F (3,146)=328.448			R2=0.871			p<0.0005	
2 mm	Constant	79.64	1.1		72.24	0	77.45	81.82
	Material	1.15	0.441	0.073	2.61	0.01	0.279	2.02
	LCU	-2.44	0.441	-0.156	-5.55	0	-3.32	-1.57
	Distance	-9.02	0.27	-0.938	-33.42	0	-9.56	-8.49
	F (3,116)=385.037			R2=0.909			p<0.0005	
Bottom	Constant	86.33	1.6		53.85	0	83.165	89.501
	Material	0.03	0.459	0.002	0.065	0.948	-0.877	0.937
	LCU	-3.19	0.687	-0.123	-4.65	0	-4.55	-1.83
	Distance	-14.94	0.421	-0.94	-35.52	0	-15.77	-14.11
	F (3,146)=427.798			R2=0.898			p<0.0005	

A quite alarming finding is the very low DC Filtek Z350 XT value seen at 4 mm LCU distance, especially at the bottom surface with the DeepCure-S despite the shorter distance of 6 mm that is to be traveled by the light, as opposed to 8 mm with bulk-fill materials. This indicates that the selection of the LCU is critical in deep cavities even with the incremental placement technique.

The results of this study confirmed that increasing the distance between the light tip and the resin composite can affect the light intensity which reaches the restorative material and can interfere with the efficacy of the polymerization, leading to weaker mechanical properties of the final restorative RBC material, especially at the deepest part of the restoration. These findings are in agreement with other studies that have stated that the effective polymerization of RBC materials is mainly dependent on the distance between the LCU tip and the restoration surface.[20,23] A similar result was reported in a recent study conducted by Ilie,[24] who concluded that bulk-fill materials did not tolerate variations in exposure distance as well as they tolerate small variations in the centricity of the LCU. Also, in this study, low VMH was shown by the Tetric N-Ceram Bulk Fill at depths larger than 3 mm, the author suggested that the lower filler content and the presence of prepolymerized particles might play a significant role.

The microhardness ratios at the top surface of all tested materials were significantly higher than the microhardness at the bottom surface in all light tip distances. This might be because more sufficient light energy reaches the photoinitiators at the top surface than at the bottom as the intensity of the light decreases while passing through the entire thickness of the bulk-fill material due to scattering and wavelength depletion. This effect was demonstrated in this study when significant differences in the DC and microhardness values were observed at a distance of 0 mm in comparison with those at 2 and 4 mm distances. However, the Filtek Bulk Fill Posterior MH values were not as significantly affected by the distances when cured with the Bluephase G2 LCU, although the variability of VMH ratios greatly increased at a distance of 4 mm. Catelan and others[25] reported that significantly lower irradiance may reach the surface of the resin in the tooth 2-8 mm away from the light tip. Moreover, the various areas of the resin might receive different amounts of light due to scattering and light attenuation, resulting in the perceived increased variability.[22] Another point to remember is that the results were reported as VMN ratios, therefore, even if the ratios remain high, the actual values might be affected by the distance and the efficiency of the curing is compromised.

The physical properties evaluated in this study are important predictors of clinical behavior of RBCs.

Microhardness enables the material to resist deformation, indentation, and scratching and predicts their resistance to abrasion and wear when used for occlusal restorations. Degree of conversion is significantly correlated to many important composite material characteristics, such as mechanical properties, volumetric shrinkage, wear resistance, and monomer elution. When the degree of conversion is low, the release of unreacted monomers from resin composite materials is high and it can induce undesirable biological responses. Therefore, utmost care must be exercised to ensure efficient curing of the resin-based restorative material particularly in deep cavity preparations.[26,27]

CONCLUSIONS

The Bluephase G2 LED LCU (polywave) revealed better polymerization efficiency than the DeepCure-S LED LCU (monowave). However, the DeepCure-S showed slightly better results in deeper areas. Increasing the distance between the LCU tip and the restoration surface was demonstrated to have a significantly detrimental influence on the mechanical properties of RBC materials. The DC and VMN values among the studied bulk-fill materials were significantly affected by the material composition and curing protocols.

Acknowledgments

The authors would like to thank the College of Dentistry Research Center and Deanship of ScientificResearch and RSSU at King Saud University, Saudi Arabia for their technical support and forapproving and funding this research (CDRC registration #PR0062). We also thank Prof. MaanAlSha'afi for his valuable contribution to this work, and the College of Dentistry physical laboratory staff for their cooperation and support.

Conflict of Interest

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

(Accepted 29 August 2021)

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Volume 47 / Number 4
July/August 2022

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