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Literature Review

Health Information Seeking and Implications for the Operative Dentist

K Walker

Clinical Relevance

This literature review provides the operative dentist with information about the demographics and psychology of the e-patient, the benefits and drawbacks of Internet use for information seeking, and tips for effectively communicating with today's e-health information seeker.

SUMMARY

The steady increase in online health information seeking by patients is ingrained in central notions of patient-centered care and shared decision-making models reflected in operative dentistry and the healthcare industry at large. More patients today seek health information prior to an appointment, communicate their findings with their providers, and expect two-way communication exchanges. This e-consumer trend has many implications for operative dentistry, for which surgery, by its very nature, lends to a confluence of questioning and informational needs. Operative dentists must acknowledge patient information and be prepared to address the breadth of information brought to them. The purpose of this literature review is threefold: 1) to provide the operative dentist with information about the demographics, psychology, and behavior of today's e-health patient; 2) to provide a review of the benefits and challenges of communicating with e-health patients; and 3) to provide recommendations for communicating with e-patients interpersonally and through Internet communication. In so doing, it is hoped that discussion can provide insight useful for improving provider/patient relationships in the progressive communication era.

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INTRODUCTION

The health consumer movement and the rise of the e-patient have led to a model of patient-centered communication and shared decision making reflected in operative dental practice and the healthcare industry at large.[1] Historically, dentists presented evidence and treatment options with a paternalistic approach, in which the dentist took full responsibility for the decision-making process.[2] The last few decades have witnessed a powerful movement toward the active, self-managing, responsible patient. More patients today seek out health information, communicate their findings to their providers, and expect two-way communication exchanges.[2]

Table 1: *Health Information Seeking Habits*
One in three American adults have searched online for health information.
Fifteen percent of those seeking health information specifically looked for dental health information.[5]
Almost 70% of individuals who turned to online resources prior to a medical visit reported they were planning to ask their healthcare provider questions about the information they found.[6]
About 40% of individuals have printed out information to take with them to discuss with their provider.[6]
More than 50% of subjects said they intended to make at least one request of their provider based on the information found.[6]

Table 2: *Patients Who Seek Internet Information and Support Preferences*
Most likely to seek information
Individuals from higher incomes and higher education brackets
Females
Non-Hispanic whites (vs non-Hispanic blacks and Hispanics)
Adults aged 18-34 y and 35-49 y (vs those 75 y and older)[9]
Patients with higher self-literacy[10]
Cultural differences in preferences for types of media and interpersonal support
Hispanics are less interested in Internet support than e-mail or telephone support before medical visits.
English- and Spanish-speaking Hispanics have lower rates of information seeking that may result from culturally based concepts of fatalism and deference to medical professionals regarding health decisions.
African Americans may be more interested than whites in a combination of Internet, e-mail, and telephone support.[10]

A key element in the shift from a more passive to a more active patient process has been the unlimited access to health-related information, particularly that which is ubiquitous on Web sites and online forums on the Internet.[3] There are more than 70,000 Web sites that provide health information for patients.[4] Table 1 presents data from the Pew Internet and Life Project Report and another large study, which shows how health information is being used.[5,6] Indeed, health-information–seeking interactions between health providers and patients have become so common that it has been recommended that courses such as 'patient informatics' be integrated into current health professionals' education.[7]

The purpose of this article is to present literature on the demographics and psychology of the e-patient, the benefits and drawbacks of Internet use for information seeking, and tips for effectively communicating with today's e-user. Although much of the research stems from the medical field, it is of relevance to operative dentistry, as dental surgery provides a critical encounter during which this professional communication takes place.[8]

What Is the Profile of a Typical Online Health Information Seeker?

Table 2 lists the characteristics of those most likely to seek and possibly bring Internet information to a consultation. It also lists cultural differences in preferences for types of media and interpersonal support, as studies indicate that the combination of race and ethnicity with language strongly influences preferences.

Where Do People Go for Online Health Information?

Consumers of health information typically access information by searching directly for health information or by participating in support groups.[4] Data from the latest Pew Research Internet Project found that most consumers (77%) accessed their health information through Google, Bing, or Yahoo, while 13% started their online health information search with centralized health sources such as WebMD, Medline, and Yahoo!health.[5] Two percent of consumers started their searches from a general site such as Wikipedia, and 1% began with a social media site such as Facebook.[5] One in four health information seekers also join a support group.[4] WebMD, Drugs.com, and MDJunction, for instance, have general oral and dental health community support groups. There are also support groups for specific oral health conditions, such as oral cancer, burning mouth syndrome, and dental anxiety. Online support groups provide a means for people to obtain advice about medical conditions, share their experiences, and seek emotional support.[4] Other advantages of online support groups have been noted, such as the 24-hour access, anonymity, selectivity in responding, capacity for immediate and time-delayed reactions, unlimited number of participants, and exposure to a larger pool of opinions, expertise, and experience that these gropus provide.[4] Acquisition of information on patient support groups has also been shown to influence medical decision making and self-management of care.[4] One study[11] found that users rated online support groups more helpful than physicians in numerous ways, including in terms of their provision of in-depth information, emotional support, and convenience.

Why Do Individuals Seek Online Health Information?

Individuals are active consumers of communication, seeking the channel of communication (Internet vs interpersonal) perceived to provide the greatest benefits for a particular task.[12] Although research of oral health communication needs is still in the primitive stages, when looking to broad patterns, the majority of people seeking online health information do so for information about disease, treatment, and procedures.[13-15] This pursuit of information is relevant to operative dentistry because research suggests that patients question dentists' behavior and attitudes most during visits in which technical procedures are performed.[16]

Communication gaps between patients and their health providers are well documented. A recent study[17] found that dentists recall more information than do their patients and report giving more dental health education and discussion of future actions than patients remember having received. Similarly, a current literature review[15] of Internet use by patients found that dissatisfaction with the amount of detailed information provided by the health provider during the medical encounter was one of the top two reasons for which patients sought online information. Patients have more confidence in dentists who they perceive communicate well. Furthermore, patients who express more confidence and trust in their dentists' communication abilities are less likely to assume an involved role in the decision-making process.[16] This is evidenced in a recent study[17] that found that patient communication behavior was negatively correlated with the number of questions patients asked during emergency consultations, indicating that the more adept the dentist is at communicating, the less information patients seek during consultation.

Although patients have more confidence in dentists who they perceive to communicate well,[16] the latest research indicates that the questioning of a dental practitioner's motivations and advice may not necessarily be due to mistrust but rather may be more of a by-product of the psychological makeup of today's online health consumer concerning three variables: 1) degree of perceived "distressfulness" of the health challenge, 2) inquisitiveness, and 3) choice.

The study of Hu and others[6] found that reliance on the Internet prior to a medical visit was not affected by level of trust in the provider, but was predicted when health situations are distressful and/or when people feel that they can have some level of personal control over their condition. The authors concluded that, "Many people go online to get information when they anticipate a challenge in their life. It makes sense that they would do the same when dealing with a health issue."

The same study[6] found that users also go online simply because they are curious and want to be more informed. Some individuals have a high need for orientation (NFO). An individual's NFO is a personality variable that reflects the extent to which individuals desire orienting cues and background information to explain the environment around them. Two variables that increase NFO are relevance and uncertainty. If individuals find the topic highly relevant and are highly uncertain of the outcomes, they experience a high need for orientation and actively seek information.[18] In other words, the higher the patient's relevance to the oral issue and the higher the patient's uncertainty about the outcome, the more likely the patient is to seek multiple communications.

A third factor that drives individuals to seek online health information is the perceived satisfaction of having choice. The belief that the provision of choice yields beneficial outcomes for both individuals and society at large is inherent in basic social science theory and research.[19] American society is guided by an assumption that the more choice one has, the greater one's well-being.[20] Choice makes most Americans feel more in control, free, and independent and thus can have positive consequences for individuals' motivation and well-being.[20] The pursuit of choice is reflected in data from a large survey concerning patients' reasons for turning to the Internet, which found that 41% go online to find information about alternative medicine, and another 41% go online to obtain second opinions about their medical condition.[21]

Pros and Cons of Information Seeking

Dual paradigms of beliefs concerning the effectiveness of health information seeking for the patient/provider relationship exist. Generally, there is now greater acceptance of the more informed and educated patient, as the "participatory" decision-making model has become the preferred model for the clinical encounter.[22] This model allows the patient to take responsibility for disclosing preferences, obtaining information, and weighing treatment alternatives.[22] Research shows that patients who seek knowledge and information for themselves report greater feelings of empowerment (belief in

ability to control one's health) and are more active in self-managing their care.[14]

From the health provider's perspective, the greatest impact on decision making may come from the increase in knowledge patients obtain prior to the clinical visit because it allows them to have opportunity to reflect on and consider preferences prior to the appointment. In this way, instead of utilizing scheduled time to provide the patient with basic knowledge, extra time can be given to refining what the patient has learned and to offering more discussion of treatment options.[22] Theoretically, prior research means more time can be spent on discussions necessary to arrive at a clinical decision. Furthermore, it has been posited that the knowledge patients bring to the appointment can make informed consent more of a reality than a theoretical concept.[22]

The above improvements in knowledge, efficiency, and treatment are dependent upon the patient's ability to access and interpret quality information. Numerous studies[23-25] have criticized the poor quality of health information on the Internet. In recognition of this, Healthy People 2020 objectives include the improvement of quality of health information on the Internet. Low health literacy rates in the United States that are dispersed among all races and ethnic groups also negatively affect the patient's ability to interpret information. The National Assessment of Adult Literacy reports that only 12% of all US adults have proficient health literacy rates.[26]

Operative dentists who are faced with inaccurate information can become frustrated and resistant as a result of the time associated with debunking myths and fallacies.[4] Given the breadth of information available, even accurate information can prompt stress and frustration when the parties involved are unprepared to deal with the magnitude of available information brought forth.[27] Conflicts may also occur if the location of information leads patients to challenge, question, or second-guess the surgeon. Most importantly, incorrect information could ultimately challenge patient care. Some fear that patients who lack technical background and who interpret information incorrectly could opt for inappropriate treatments, reducing medical outcomes.[28] Furthermore, in the pressure to embrace patient-centered care, a provider may acquiesce to requests that may make the patient happy but are not necessarily what he needs.[29] A recent national survey of 3500 physicians, for instance, found that 43% of physicians in practice more than 30 years sometimes or often gave in to patients' demands for brand-name drugs because the patient wanted it, even though a generic is available.[30]

Ways to Communicate More Efficiently

Despite potential drawbacks, the trend of consumer health information seeking is only increasing. Given the nature of technical information required during surgical proceedings, operative dentists are likely to remain at the crux of Internet exchanges and provision of care. The following are tips to help operative dentists effectively communicate with today's e-net user.

Listen—When e-information is brought forward, try not to respond defensively and assert an expert opinion without listening.[15] In a recent survey, one-third of medical patients who felt their relationship with their physician was "low" in participatory decision making changed providers within a year.[31] Communication skills are one of the most important features by which patients judge their dentists.[32] In addition to technical knowledge, patients expect their dentist to communicate effectively, which is specifically defined by use of active listening skills and the effective use of gathering and imparting of information.[33] It is imperative today to acknowledge patients' search for knowledge and to provide some discussion of the information offered.

Guide Patients to Reliable Health Web Sites—Once information is acknowledged, take advantage of the opportunity to create, support, reference, and promote awareness of quality electronic sources of medical information. Consider guiding patients to reliable Web sites in two ways: by providing a tip sheet to help patients understand and evaluate Web site credibility and by providing a list of specific Web sites to read. Templates of credibility fact sheets such as those found at the US Food and Drug Administration can be used as models within the dentist's own practice. The US Food and Drug Administration addresses questions that consumers should ask to evaluate the credibility of information found on the Internet, including who runs the Web site, the purpose of the Web site, the original source of information on the Web site (.gov, .edu, .org), review of credentials, timeliness, and the Web site's linking policy.[34] Additionally, dentists could prepare a list of Web sites that they trust and share it with their patients. Web sites sponsored by the government, academic medical centers, or professional medical societies typically have authoritative information that can be relied upon.[35] A leading

healthcare provider[36] recommends that medical professionals start prescribing the right sites to be used for further information about the patient's condition, and this can be done for operative dentistry patients as well. The list would not only provide authentic material to the patient but would also save the patient time.[36]

Prepare Written Materials—Consider preparing written materials about commonly asked procedures, especially those for which patients have little knowledge or are misinformed about. There are some questions that arise over and over that stem from natural anxieties and lack of knowledge about surgical procedures. Take these questions and create brochures and other written materials addressing them. Research shows that up to 80% of information given during consultation is forgotten by the patient, indicating a functional need for both the patient and the dentist.[37] "A Patient's Guide to Orthognathic Surgery" is an example of an article written to give information related to commonly misconstrued ideas about orthognathic surgery, its purposes, and complications.[38] The Centers for Disease Control and Prevention offers practical tips for writing clear health communications.[39]

Use Social Media—The external media environment also places "trending" health issues and behaviors on the public agenda with stories and anecdotal evidence that can quickly become salient to the public. To counteract popular trending fallacies, consider meeting patients where they are on social media. Take examples from hospitals like the Mayo Clinic, which have well-established social media networks. Dr. Farris, Assistant Professor of Medicine and Medical Director for the Mayo Clinic Center for Social Media, believes that the provision of correct information for the mass audience is more of a moral obligation than a function of efficiency. The popular misconception that vaccinations do more harm than good that has been cultivated by media celebrities is an example of an issue he feels morally obligated to counteract. In an interview with the author, he said that he spends approximately 10 minutes per visit talking about the rationale for vaccinations with his patients and has approximately one refusal a month. The impact of even one vaccine refusal a month can be catastrophic to healthcare, and when individuals become ill, they often sue. Bike helmet safety is another issue that he says pediatricians talk about at least 15 times a week. Posting a simple, short video demonstrating bike safety has saved consultation time and advanced provision of care.

Speak In Loss-framed Language—For the impenetrable patient bent on information deemed contradictory to best surgical practice, speak in loss-term language. In health communication, a loss-frame refers to phrasing an argument in terms of the consequences that will occur if a behavior/treatment is not undertaken.[40] For example: If you do not have orthognathic surgery, you may have masticatory insufficiency. Prospect theory research in the health arena demonstrates individuals tend to be more inclined to risk taking with behaviors involving detection and high risk (eg, surgery) when the discussions are positioned in terms of what will be lost.[41] On the other hand, individuals tend to be more motivated to perform preventive behaviors (eg, brushing/flossing) when conversations are framed in terms of the gains they obtain from performing the behavior (strong, clean teeth). If it is a detection/surgical behavior deemed in the patient's best interest, speak in terms of possible losses acquired by not following the procedure.

CONCLUSION

The trend of seeking online health information has many implications for operative dentistry, which by its very surgical nature creates a confluence of questioning and informational needs. Operative dentists must acknowledge patient information and be prepared to address the breadth of information and misinformation brought to them.

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.

Note

This study was conducted at the Indiana University School of Dentistry.

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Clinical Technique/Case Report

Use of a Copper Band to Make Resin Cores in Endodontically Treated Teeth Lacking Coronal Structure

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Clinical Relevance

We consider the use of a copper band as the most suitable technique for isolating an endodontically treated tooth that has a subgingival margin, while the band also serves as a matrix to fabricate the core.

SUMMARY

This article describes the use of a copper band as a matrix to build up resin cores in endodontically treated teeth that have a partially subgingival margin. The copper band is adjusted to the contour of the remaining dental structure and extends beyond the margins to ensure complete isolation in order to provide a matrix to fabricate a core.

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PURPOSE

It has been suggested that a minimum ferrule of more than 1.5-2 mm is necessary to guarantee the success of restorations for endodontically treated teeth.[1,2] The ferrule length is affected by the biologic width[3] because the maintenance of the biologic width is essential to the health of the supporting tissues.[4] In addition, when one wall of remaining tissue is conserved, there is a reduced risk of failure.[5]

When a fiber-reinforced composite post and a core are required as a result of extensive loss of natural tooth substance, isolation of the operative field must be performed to avoid contamination, especially when using an adhesive technique.[6]

The current authors consider the use of a copper band as the most suitable technique for isolating an endodontically treated tooth that has a subgingival margin, while the band also serves as a matrix to fabricate the core. Copper bands (E. Hahnenkratt GmbH Dentale, Königsbach-Stein, Germany) are available in several diameters, ranging from 5 to 12 mm, and can be hard or soft. The current authors prefer the use of hard copper bands.

The current literature describes the use of a copper band to take impressions,[7] make direct

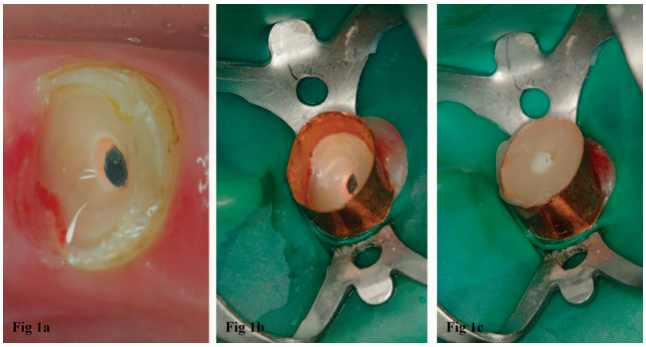

Figure 1. *(A) Tooth with a mesial subgingival margin. (B) The copper band allows for the isolation of the operative field with a clamp and rubber dam. (C) Matrix filled with Core•X flow (Dentsply Core & Post System. Dentsply Detrey GmbH, Konstanz, Germany).*

restorations,[8] and isolate the operative field in extensively damaged teeth.[9-11]

This current article describes the use of a copper band as a matrix to build up resin cores in endodontically treated teeth with subgingival margins.

CLINICAL TECHNIQUE

Prior to initiating treatment, it is necessary to evaluate the possibility of restoring a tooth without invading the biologic width.[12] In addition, it is important to take into account whether the prognosis is better if the tooth is restored immediately after endodontic therapy in order to avoid coronal microleakage.[13-16]

In endodontically treated teeth that have subgingival margins, the use of a clamp will impede the correct fit of the matrix on the tooth. In contrast, the use of a copper band, which fits properly below the subgingival area, allows the tooth to be isolated without the need for a rubber dam. Additionally, a clamp can be placed on the tooth after the copper band is placed if a rubber dam is desired to isolate the field (Figure 1).

Prior insertion of the copper band is necessary in order to create a bevel in the infragingival margins and to facilitate the insertion of the copper band.

The selection of the proper diameter of copper band is an important step in this procedure. The copper band should be precisely adjusted to the contour of the remaining dental structure and extend beyond the margins to ensure complete isolation. In those teeth in which the tooth is oval shaped, it is advisable to ensure that the copper band will adapt to the contour of the tooth.

In cases in which there is a remaining wall of dental tissue, the copper band must be cut, following the shape of the margin, to ensure it extends to the subgingival areas. The copper band is then trimmed with curved-tip scissors for cutting metal until its height corresponds to that of the future core, using the height of the adjacent teeth as a guide. The prepared copper band is then seated by applying sufficient vertical pressure. If the operator desires to use thumb pressure, a cotton roll can be interposed between the thumb and the copper band to avoid injury to the thumb. On occasions when more force is required, the patient can be instructed to bite on the cotton roll so that the copper band is seated beyond the margins of the tooth.

In order for the placement of the copper band to be appropriate, it must extend beyond the subgingival margins and be stable. Although insertion of the band can cause gingival bleeding, the blood should not penetrate inside the copper band because the purpose of this technique is to generate an isolated operative field, allowing for the use the adhesive technique to place a post and core under aseptic conditions.

Often, the insertion of the matrix causes some gingival tissue to remain inside the operative area as a result of the cutting effect of the copper band. A dental probe can be used to eliminate this gingival tissue and to confirm the correct placement of the matrix.

Once the tooth is isolated, the post and core are prepared following the manufacturer's recommendations. Once the post and core are fabricated, the copper band is removed by creating a vertical cut along the full height of the copper band with a diamond bur (Figure 2).

Once the post and core are completed, the tooth can be prepared for a crown with margins in healthy dental tissue (Figures 2 and 3),[17] and a temporary crown is placed to assess any changes in the tooth and soft tissues for a few weeks.[18]

SUMMARY AND ADVANTAGES AND DISADVANTAGES

This technique is useful for restoring endodontically treated teeth when part of their structure is subgingival.

Advantages

A copper band is the only matrix that can be shaped intimately to the dental structure in the subgingival area. Copper band isolation allows optimal condi-

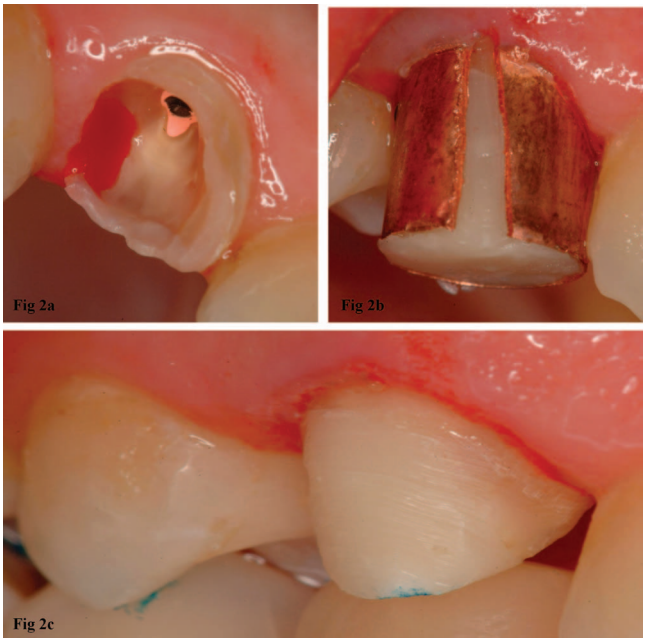

Figure 2. *(A) Upper canine with a distal subgingival margin. (B) A vertical cut made in the copper band to enable its removal. (C) The margins of the final preparation in dental tissue.*

tions for the adhesive technique, allowing for a direct post and core buildup in teeth with an irregular ferrule.

Unlike other preparations, such as cast-metal post and cores, the use of a copper band does not require removal of healthy dental tissues during preparation.

Disadvantages

This technique depends on the clinician's skill. It can be difficult to shape the matrix for teeth with irregular forms, such as the interproximal concavities of maxillary premolars, which would require the placement of interproximal wedges.

Human Subjects Statement

This report was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the School of Dentistry Santiago de Compostela, Spain.

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 20 January 2015)

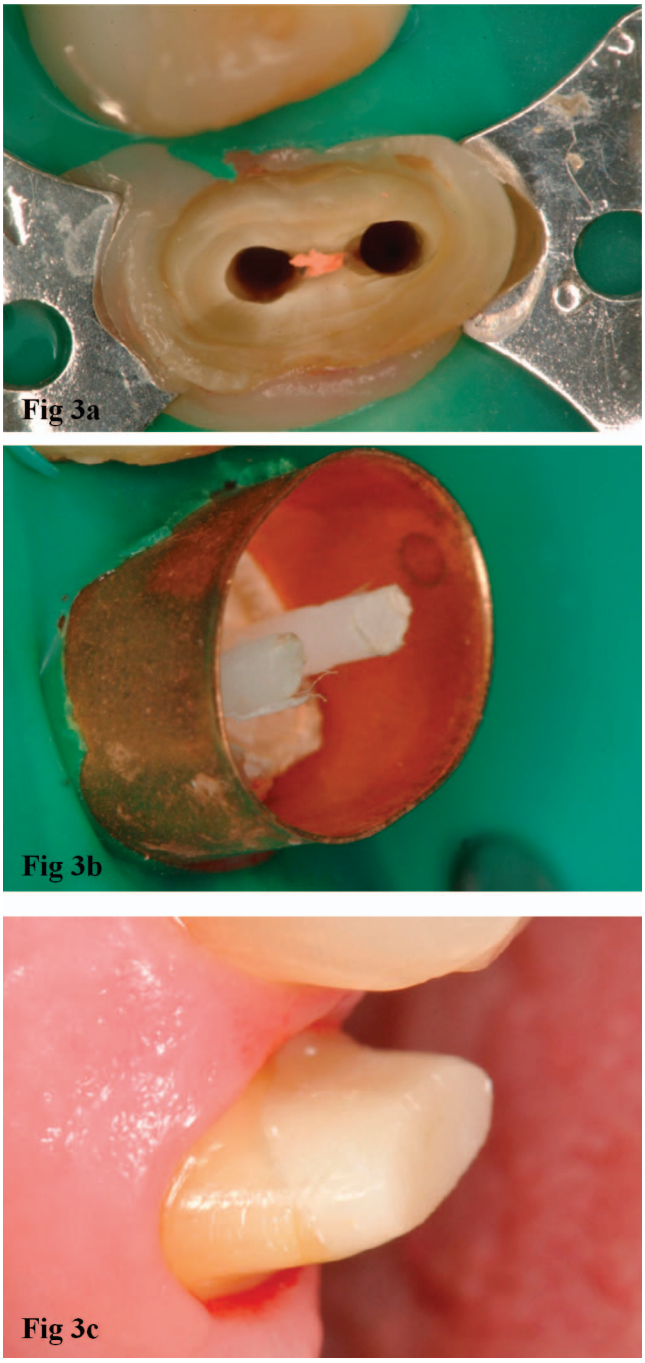

Figure 3. *(A) In this case, the post space preparation was performed before placement of the copper band. (B) After placement of the matrix, the posts (Rebilda Post System; (VOCO GmbH, Cuxhaven, Germany) were cemented using the adhesive technique. (C) Resin core (Rebilda Post System; VOCO GmbH, Cuxhaven, Germany). The preparation for placement of a crown had margins in healthy tissue.*

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Conservative Restoration of Worn Mandibular Anterior Teeth Combining Gingival Repositioning and a Template Matricing Technique

HA St Germain Jr • JF Jenkins

Clinical Relevance

The restoration of worn mandibular anterior teeth is a challenging clinical problem. By combining gingival crown lengthening, bonding of resin composite material, and selective occlusal adjustment, a short to medium-term, conservative option can be made available for patients.

SUMMARY

Conservative resin composite restoration of worn mandibular anterior teeth may offer an alternative option to full-coverage restorations for the patient. Assessment of the occlusal condition is critical because alterations in occlusal vertical dimension may not always be possible. By exposing additional coronal tooth structure, periodontal crown-lengthening procedures can serve to increase clinical crown height when adequate attached gingival tissue is present and supra-eruption has likely occurred. Fabrication of a custom template made from a diagnostic mock-up with proximal stainless steel matrices helps contribute to a predictable restorative result and improves chairside efficiency for the dental practice. By combining gingival crown lengthening, bonding of resin composite material, and selective occlusal adjustment; a short to medium-term, conservative option can be made available for the patient.

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INTRODUCTION

The retention of natural teeth in an aging population, accompanied by the loss of posterior teeth, parafunctional habits, and tooth inclines (natural or

restorations) that interfere with mandibular border paths, can result in loss of incisal or occlusal tooth structure in the adult dentition.[1,2] Restricted anterior guidance resulting from orthodontics or improperly contoured anterior restorations can cause excessive wear, hypermobility, and migration of anterior teeth.[2] When mandibular anterior wear is present, the enamel is often worn coronally such that the dentin is exposed. The exposed dentin will erode faster than enamel because it does not resist abrasion as well and demineralizes at higher pH levels. Many times sharp, translucent ledges of enamel are left unsupported by dentin, and these are prone to fracture and have an appearance that is esthetically displeasing to the patient.[3,4]

For patients in whom functional occlusion mainly involves mandibular movement in an anteroposterior direction, the anterior teeth abrade slowly as they continue to erupt to maintain a maximum intercuspation position (MIP). Orthodontic intrusion may be necessary to create space for a definitive restoration, such as an all-ceramic crown or porcelain veneer.[5] However, it may not be desirable to increase the occlusal vertical dimension (OVD) in patients who are bruxers as this may result in premature destruction of the restorations.[6] Orthodontic treatment, however, may take significant time and requires close coordination between the restorative dentist and the orthodontist. A commonly recommended restorative solution for tooth wear involves increasing the OVD, which serves to provide space for the full-mouth restoration of the worn anterior teeth.[7] Restoring the severely worn dentition or a dentition with a deep bite is challenging when space for the restorations is inadequate.[8] Increasing the OVD to create space for restorations has proven successful in full-mouth rehabilitation; however, conservative increases (<5.0 mm) of OVD must be trialed with temporary restorations or occlusal splints for up to three months with the patient reporting no signs or symptoms of muscle soreness or temporomandibular joint pain before completing definitive restorations .[9,10] Although clinical strategies for full-mouth restoration of severely worn teeth that conservatively increase the OVD have been successful, some retrospective case studies have suggested that the prognosis and adaptability of increasing OVD in adults is not predictable and that patients tend to return to their original OVD.[11,12]

Because orthodontics and indirect restorations often involve time-consuming and expensive procedures; patients will request alternative options for restoring their worn teeth. Recently reported case studies with a clinical follow-up that evaluated the reconstruction of a complete occlusion with resin composite materials presented longevity results after three years and after five and a half years.[13,14] A vacuum-formed template was used to build up the teeth with resin composite materials to optimal anatomy and function. Out of a total of 85 restored teeth, two teeth sustained small restoration fractures after 24 months, though these were repairable. At five and a half years these build-ups showed some deterioration, but most of the marginal defects could be corrected by finishing and polishing, and the authors suggested that this approach could serve as a "medium-term" restorative rehabilitation for patients.

In another study, direct composite restorations were placed on mandibular anterior teeth for patients who wore a removable posterior occlusal overlay appliance that stabilized posterior contacts at an increased OVD.[15] Based on the results after two and a half years, the authors suggest that direct composite restorations are a "simple and time-efficient" technique to manage a worn mandibular anterior dentition but do not imply that this is a "long-term" solution. Recently, researchers from this same clinical trial published a seven-year follow-up on the longevity of the direct composite restorations.[16] Marginal breakdown was the most frequently encountered problem, but at the seven-year evaluation, 85% of the restorations remained intact and functional.

Although other studies[17,18] advocate the use of an occlusal splint to increase OVD when restoring worn anterior teeth with direct composites, selective occlusal adjustment and maintenance of the existing OVD may be desirable when restoring worn teeth with resin composite materials.[19] The use of a prefabricated template has been suggested to achieve a predetermined contour and occlusion when using direct-placement resin composite materials.[20-22] Also, worn mandibular teeth may have supra-erupted to stay in occlusion, in which case the clinical crown may then be further exposed using crown-lengthening repositioning surgery to create additional crown height and improve the esthetic appearance of the teeth.[23] This supra-eruption has been termed "delayed passive eruption" and is associated with excess gingiva covering the anatomic crown, resulting in the appearance of short teeth.[24] In these cases, the osseous crest may be close to the cementoenamel junction (CEJ), and osseous resection will be indicated in combination with soft tissue repositioning

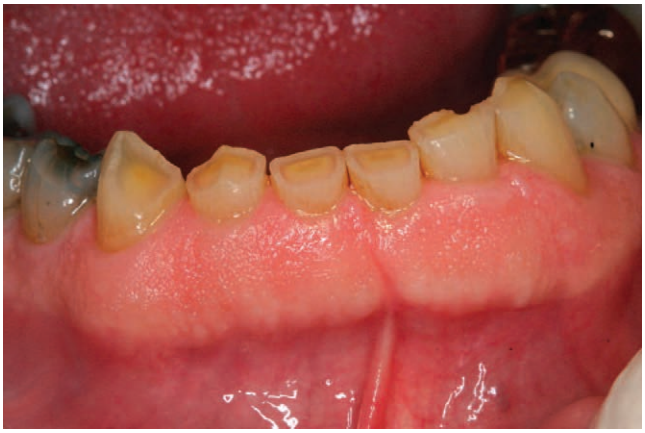

Figure 1. *Preoperative condition of worn mandibular anterior teeth.*

typically involving gingivectomy and/or flap surgery.[25]

This clinical technique article will present a conservative restoration combining gingival crown lengthening and restoration of worn incisal edges with resin composite material using a unique prefabricated template for worn mandibular anterior teeth in the patient's acquired MIP.

CLINICAL TECHNIQUE

A 72-year-old man presented to our dental college faculty practice seeking treatment options for his worn mandibular anterior teeth. His medical history was noncontributory, except for taking medication for hypertension. The clinical exam revealed the following: all maxillary molars, mandibular third molars, and tooth #10 were missing, generalized marginal gingivitis with mild horizontal bone loss, gingival probing depths $\leq$4 mm, severe wear with dentin exposure and sharp edges of enamel on teeth #23–27, extensive amalgam restorations, porcelain fused to metal (PFM) crowns on teeth #7 and #8, and a three-unit PFM fixed partial denture (FPD) with abutments on teeth #9 and #11. The patient reported no history of bruxism, and the temporomandibular joint function was asymptomatic with no joint noise or masticatory muscle tenderness. The patient's spouse was not aware of nocturnal bruxism. The missing maxillary molar teeth were removed due to nonrestorable caries and tooth fractures that occurred when the patient was in his 30's. At the time of the current exam the patient's caries risk status was moderately high due to a high decayed, missing, and filled tooth index (DMFT), lack of access to water fluoridation, fair oral hygiene, and the use of prescription saliva-reducing medications; however, no active caries lesions were present. The patient was not in any discomfort and was referred to the faculty practice for full-mouth reconstruction to restore his worn dentition.

Diagnostic casts were fabricated, a face-bow recording was obtained, and the casts were mounted on a semiadjustable articulator in centric relation (CR). This diagnostic procedure revealed that the patient was functioning in an acquired protrusively positioned MIP that was likely an etiologic factor for incisal wear along with the mandibular tooth enamel/dentin occluding against maxillary PFM restorations. The PFM crowns on teeth #7 and #8 and the fixed partial denture from #9–11 had a lingual metal contact design opposing the incisal edges of the mandibular anterior teeth. An initial treatment plan involving reconstruction of the complete dentition with full-coverage restorations, crown-lengthening surgery for the mandibular anterior teeth, and implant restorations for the missing maxillary molars was presented to the patient.

Because of concerns about cost and time, the patient requested a secondary option. The alternative proposal involved crown-lengthening periodontal surgery for the mandibular anterior teeth followed by resin composite restoration of the incisal edges of teeth #23–27 combined with selective grinding of the lingual metal surfaces of the maxillary fixed prostheses. The rationale for restoring teeth #23–27 was to restore the worn incisal edges to an improved incisal anatomy, remove the sharp enamel edges, and to protect the exposed dentin surfaces from further loss of tooth structure. Although this treatment proposal was a compromise from the preferred reconstruction, the patient accepted this plan and understood that this would not be a long-term solution for preserving his dentition. Probing the facial aspects of the mandibular anterior teeth revealed that the CEJ was $\geq$2.0 mm from the gingival margin, which suggested that these teeth had delayed passive eruption in addition to abrasive/erosive wear. The amount of facial attached gingiva was abundant and measured $\geq$4.0 mm from the free gingival margin to the mucogingival junction on the facial aspect of teeth #23–27.

The extent of the tooth structure loss on the mandibular anterior teeth is depicted in Figure 1, and the two-week healing of the periodontal crown-lengthening surgery is illustrated in Figure 2. Although the crown-lengthening procedure resulted in apical positioning of the gingiva and increased incisogingival length of the incisors, it did not expose the CEJ. The restorative procedures were completed

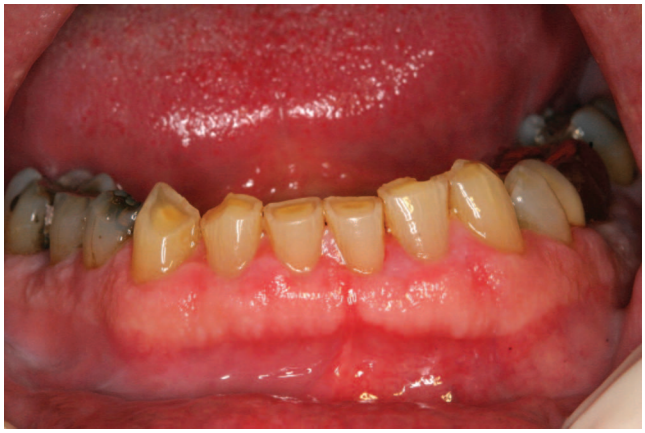

Figure 2. *Two-week postoperative healing after crown-lengthening surgery of the mandibular anterior teeth.*

six weeks after the periodontal surgery. Following are the steps taken in the process.

Step One

A diagnostic mock-up using expired resin composite for teeth #23–27 was accomplished on a simple hinge articulator mounted in the patient's acquired MIP because restoration to the CR position would require replacement (either fixed implant or removable prostheses) of the missing maxillary molars and restorative establishment of a stable posterior closure coincident with CR. In addition to contouring the mock-up to avoid excessive occlusal contact, some selective grinding on the diagnostic cast of the maxillary anterior restoration lingual surfaces on metal was performed to accommodate a symmetrical esthetic result for the incisal edges of the mandibular teeth within the constraints of the patient's

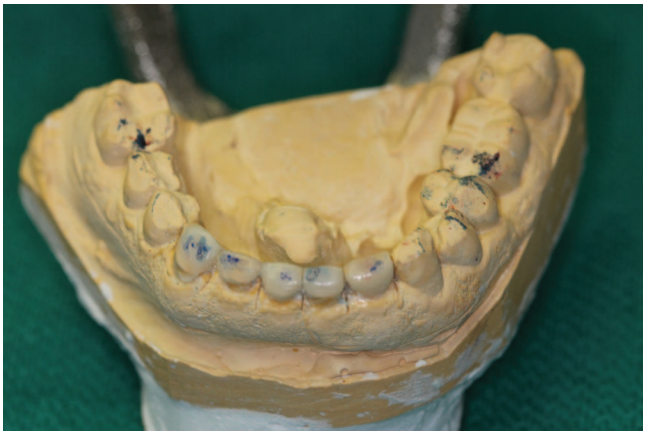

Figure 3. *Model with the diagnostic mock-up on the mandibular anterior teeth.*

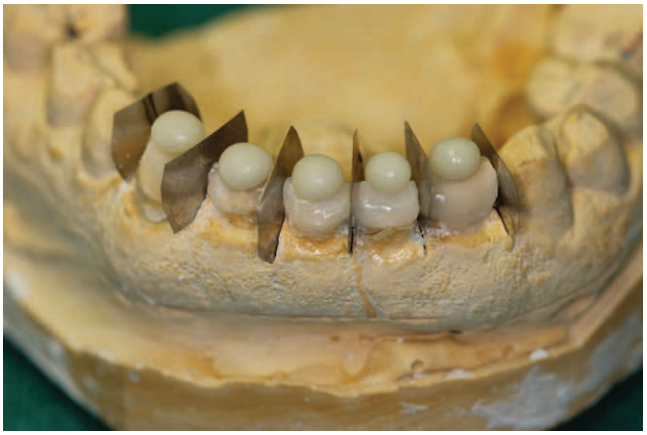

Figure 4. *Proximal positioning of the stainless steel matrices and the placement of a round bead on the incisal edge to create a sprue for the resin composite tip.*

acquired occlusal scheme. There was a risk of perforating through the metal on the maxillary PFM prostheses to the underlying tooth structure or buildup material; however, because the selective grinding was minimal, we were able to avoid perforating the existing restorations. The resin composite mock-up is shown in Figure 3. The OVD was not changed, so the incisal length of the mandibular anterior teeth was limited by the MIP and the patient's anterior guidance.

Step Two

Figure 4 illustrates the custom template preliminary procedures on the study model after the diagnostic mock-up was completed. Proximal cuts were made with a Ceri-saw (DenMat Holdings, Inc, Lompoc, CA, USA) to open up the interproximal areas on the stone cast. Round beads of resin composite of similar diameter to the tip of the resin composite applicator were placed on top of teeth #23–27, serving as a sprue, and sections of stainless steel (SS) matrix material (0.0015" #1 Tofflemire matrix, Safco Dental Supply, Buffalo Grove, IL, USA) were placed mesially and distally of each tooth being restored to avoid inadvertent placement of resin composite on adjacent teeth.

Step Three

A template (0.020" clear coping material, Patterson Dental Supply, Inc, Effingham, IL, USA) was then fabricated using a vacuum-former (UltraVac, Ultradent Products, Inc, South Jordan, UT, USA) that embedded the diagnostic mock-up and SS matrices. The sections of SS matrix material were removed, and these areas were opened up buccolingually with

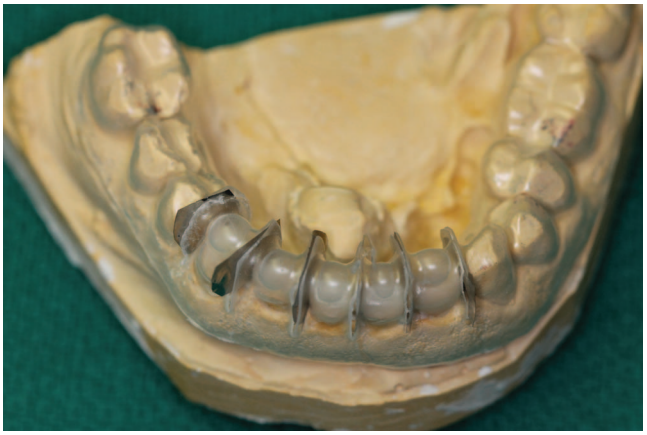

Figure 5. *Template seated on the diagnostic cast mock-up with stainless steel matrices in place after opening up the interproximal space on the template with a #25 blade.*

a #6 Bard-Parker scalpel (Zahn Dental/Henry Schein, Cookstown, NJ, USA) with a #25 blade to facilitate subsequent placement of the SS matrices back in place when the template is transferred to the mouth. Figure 5 shows the custom template with the SS interproximal matrices in place. [27]

Step Four

Figure 6 shows the preparation design for teeth #23–27 with two retentive potholes at the incisal edge, 1.0 mm in diameter and 1.0 mm deep, in dentin using a #329 carbide bur (Brasseler USA, Savannah, GA, USA), and the peripheral enamel was prepared with a circumferential 1.0-mm bevel using a #8889 ultra-thin flame diamond (Brasseler USA). Proximal relationships between the mandibular anterior teeth were left as they existed preoperatively.

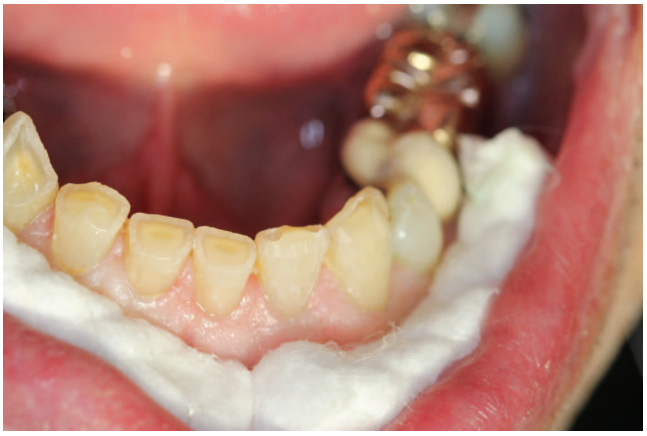

Figure 6. *Example of a tooth preparation on the mandibular left lateral incisor illustrating retentive potholes and the peripheral circumferential bevel.*

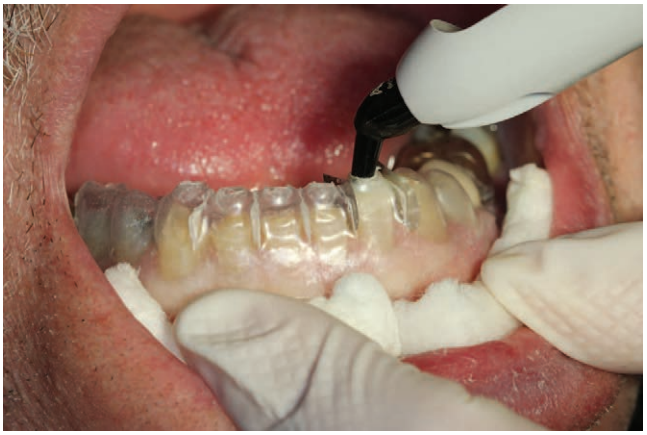

Figure 7. *Custom template in place intraorally with interproximal stainless steel matrices and resin composite tip placed in the sprue hole for building up one tooth at a time.*

Step Five

Figure 7 illustrates the interproximal SS matrices in place and the positioning of the resin composite delivery tip for filling the custom template and building up the restoration. The teeth were etched with 35% phosphoric acid, a two-step primer & bonding resin application and followed by restoration with a radiopaque, microhybrid resin composite material. The brand names of the materials used were Ultra-etch, PermaQuick, and Amelogen Plus (Ultradent Products, Inc). The teeth were built up one at a time. Etching and placement of the primer and bonding resin were accomplished before placement of the custom template. The resin composite buildup required only one increment, which was $\leq$ 2.0 mm, so incremental light polymerization was not needed. The resin composite delivery tip was placed close to the surface of the tooth inside the access hole in the custom template. While slowly being with-

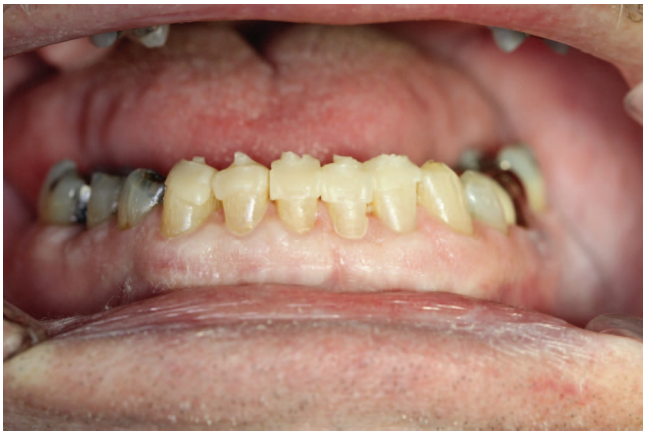

Figure 8. *Mandibular anterior teeth with resin composite buildups ready for contouring and polishing.*

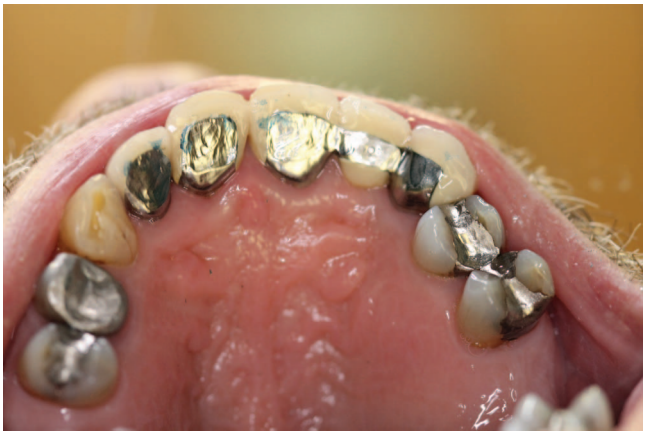

Figure 9. *Selective grinding completed prior to final finishing on the lingual metal of the maxillary fixed prostheses.*

drawn, pressure was generated to force the resin composite into the template space, resulting in a slight amount of excess composite requiring minimal contouring and finishing. The resin composite sprue served to minimize air voids in the buildup. Figure 8 shows the completed buildups before finishing and polishing. The sprue button on the top of the incisal edge was carefully finished to the predetermined diagnostic mock-up incisal edge configuration, and the proximal embrasures were finished to remove excess composite.

Step Six

The patient's occlusal contacts were in harmony with MIP and protrusive guidance with maintenance of the preexisting OVD. Figure 9 illustrates the lingual metal surfaces of the maxillary fixed prostheses

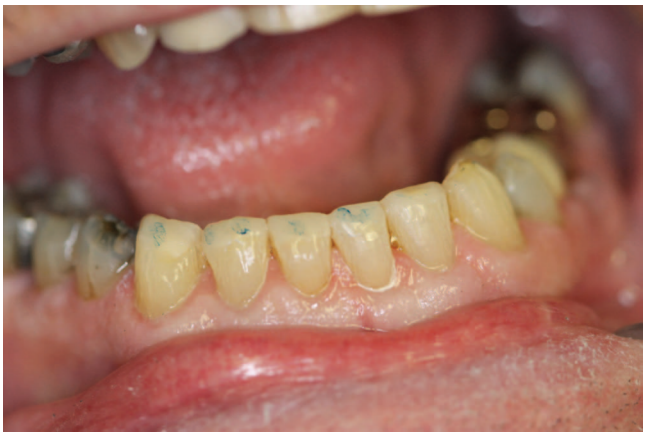

Figure 10. *Final occlusal markings on contoured and polished resin composite restorations on the mandibular anterior teeth. Minimal adjustment was necessary, and the resin composite restorations were subsequently polished to a high gloss with abrasive-impregnated rubber points, cups, and disks.*

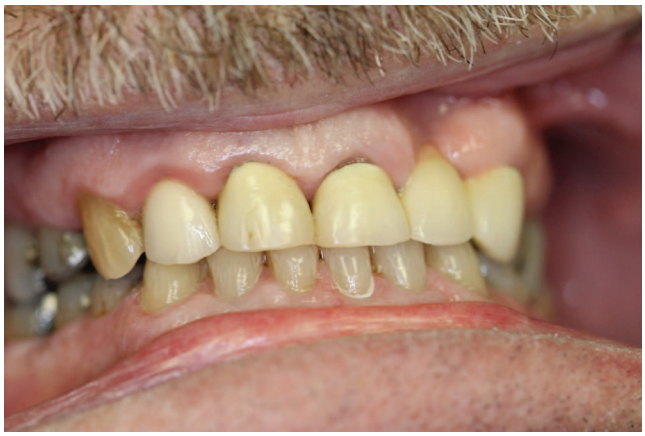

Figure 11. *Frontal perspective of the patient in maximum intercuspation position after gingival surgery and resin composite restorations.*

immediately after the selective grinding was accomplished using the occlusal adjustment planning done on the diagnostic cast as a guide. These rough surfaces were subsequently smoothed using rubber abrasives. Since compromises were made in the incisal anatomy on the diagnostic mock-up to minimize increases in OVD, perforation of metal wasavoided, and the lingual metal surfaces were conservatively contoured as planned, finished, and polished. Figure 10 shows the final results of the resin composite restorations after finishing, polishing, and checking MIP and anterior guidance contacts and the healthy gingival tissues. A frontal perspective of the patient in MIP is depicted in Figure 11.

Step Seven

The patient agreed to comply with six-month recall visits for continued hygiene maintenance and restorative examination. Although a plan for a comprehensive full-mouth rehabilitation was suggested to the patient at the recall visits, his financial situation would not allow him to proceed with this level of care. A maxillary acrylic occlusal splint was fabricated for the maxillary arch at the first six-month recall for nighttime use, although the patient (and his spouse) continued to deny any awareness of nocturnal bruxism. If bruxism were contributing to the wear of his mandibular anterior teeth this would likely lead to early failure of the resin composite restorations and continued wear of tooth structure would be observed.[6]

At the two-year recall visit in January 2014, it was observed that the resin composite restoration on tooth #27 had sustained a partial cohesive fracture. The preparation was modified to increase retention,

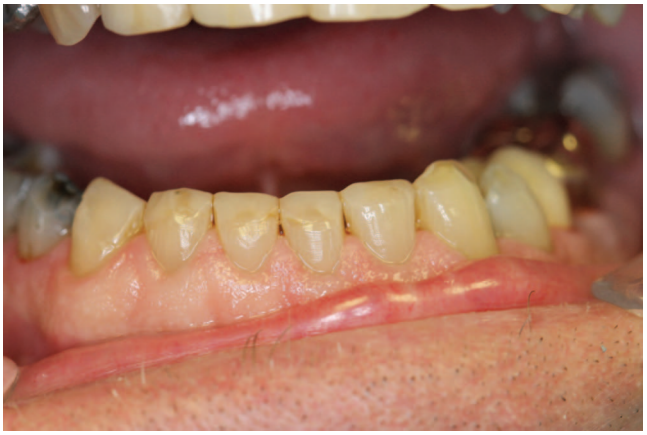

Figure 12. *Three-year post-operative condition of mandibular anterior teeth with resin composite build-ups.*

the peripheral bevel was prepared again, and the tooth was subsequently restored to the previously established contour and occlusion on the diagnostic mock-up with resin composite using the original custom template with interproximal matrices. Figure 12 illustrates the condition of the restored mandibular anterior teeth after 3 years. These restorations continue to be retained although there are signs of wear on the resin composite material. The maxillary occlusal splint required no modifications.

SUMMARY

Worn mandibular anterior teeth are commonly observed in members of an aging population who are partially edentulous as well as those who maintain a full permanent dentition. Occasionally, increasing the OVD of the posterior teeth is necessary to create space for the restoration of worn anterior teeth. Also, the situation may present where excess gingival tissue covers the anatomic crowns of the worn anterior teeth. In such cases, soft tissue repositioning using gingivectomy and surgical flap procedures will expose additional clinical crown, thereby improving esthetics; however, the incisal edges of these teeth are typically worn and uneven. A diagnostic mounting in MIP on a simple hinge articulator facilitated a mock-up on the diagnostic cast for the incisal edge restoration with resin composite restorative materials to serve as a short- to medium-term restorative option. Subsequently, fabrication of a clear custom template with interproximal SS matrices will serve to minimize time required for chairside buildup and contouring of the direct placement resin composite restorations, thereby enhancing clinical efficiency for both the practitioner and the patient.

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Human Subjects Statement

This study was conducted in accordance with all the provisions of the local human subject's guidelines and policies of the University of Nebraska Medical Center College of Dentistry.

Conflict of Interest

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

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Laboratory Research

The Effect of Hydrofluoric Acid Concentration on the Bond Strength and Morphology of the Surface and Interface of Glass Ceramics to a Resin Cement

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AB Correr • S Consani • GA Borges
L Correr-Sobrinho

Clinical Relevance

The current results support that 7.5% and 10% concentrations of hydrofluoric acid are more reliable for etching glass ceramics than are higher or lower concentrations. The use of unfilled resin after silane resulted in higher microshear bond strength and provided better interaction between ceramic and resin cement.

SUMMARY

The purpose of this study was to evaluate the influence of various concentrations of hydrofluoric acid (HF) on the surface/interface morphology and μ–shear bond strength (μSBS) between IPS Empress Esthetic (EST) (Ivoclar Vivadent) and IPS e.max Press (EMX) (Ivoclar Vivadent) ceramics and resin cement. Ceramic blocks were divided into 12 groups for each

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kind of ceramic. Six different HF concentrations were evaluated: 1%, 2.5%, 5%, 7.5%, 10%, and 15%. All groups were silanated after etching, and half of the specimens within each group received a thin layer of unfilled resin (UR). Three resin cement cylinders were prepared on each ceramic block for μSBS testing. The specimens were stored in distilled water at 37°C for 24 hours. The μSBS test was carried out in a universal testing machine at a crosshead speed of 0.5 mm/min until fracture. The data were submitted to three-way analysis of variance and multiple comparisons were performed using the Tukey post hoc test ($p<0.05$). The etched surfaces and bonded interfaces were evaluated using scanning electron microscopy. μSBS means (MPa) for 1%, 2.5%, 5%, 7.5%, 10%, and 15% HF concentrations were, respectively, 25.2, 27.2, 30.1, 31.4, 33.3, and 31.8. μSBS means with or without UR application measured 32.24 and 27.4, respectively; EST and EMX measured 29.8 and 29.9, respectively. For the HF concentrations, 10% and 15% showed higher μSBS means than did 1% and 2.5% ($p<0.05$); 7.5% was higher than 1% ($p<0.05$); and no statistical differences were found among the other concentrations ($p>0.05$). When evaluating UR, μSBS mean was significantly higher and better infiltration was observed on the etched surfaces. No statistical difference was found between the ceramics. The HF concentration and UR influenced the bond strength and surface/interface morphology.

INTRODUCTION

The indications for using dental ceramics have increased as a result of their optimal characteristics, such as their ability to mimic the function and esthetics of dental tissues, biocompatibility, color stability, high mechanical resistance, radiopacity, and low thermal conductivity.[1] Among the several types of ceramics used in dentistry, IPS e.max Press (EMX; Ivoclar Vivadent, Schaan, Liechtenstein), which contains lithium disilicate and is reinforced with lithium orthophosphate crystals,[1] and IPS Empress Esthetic (EST; Ivoclar Vivadent), a leucite-reinforced material, can be highlighted, as they are widely utilized and have been evaluated in many studies.[2]

The bond between glass ceramics and resin cements is one of the key factors to long-term clinical success.[3] Additionally, the bonding quality has a direct relation to the ceramic type involved, as well as to the variables that influence ceramic surface etching. Etching is responsible for both an increased contact surface area and improving the interaction between the luting agent and ceramic.[4,5] The size and number of irregularities created on the surface of a glass ceramic as a result of etching have been associated with acid formulation, dilution of the acid,[4,6] and etching time.[5,7-9] Researchers have reported that the EMX surface should be conditioned for 20 seconds[10] and the EST surface for 60 seconds, in both cases with 10% hydrofluoric acid (HF) as a chemical surface conditioner.[11-15] HF requires minimal time for effective application, carries a low cost, and is very efficient at creating surface roughness.[16,17]

On the other hand, HF acid is extremely corrosive and is capable of causing severe trauma to soft tissues. Furthermore, the lesion severity is directly related to the exposure time and acid concentration.[15] Even though HF is not normally applied on soft tissue, less concentrated HF would cause less injury in accidental contact situations. Little is known about the effect of increased or decreased HF concentrations on the surface morphology or bonding ability of this ceramic. Trakyali and others[18] showed that no statistical difference was found in terms of bond strength when using HF concentrations of 5% and 9.6%.

Resin cements need sufficient wettability to completely infiltrate the irregularities of a ceramic surface.[19,20] Normally, manufacturers recommend the use of silane on the internal ceramic surface prior to applying resin cement. With the addition of silica, glass ceramics are able to be adhesively bonded, with chemical bonding to the resin cement due to the previous application of silane, which improves the durability and bond strength.[11,13,21-24] However, it is questionable if the silane and resin cement are efficient in wetting the surface and filling up irregularities when different HF concentrations are used. Although some clinicians apply a layer of unfilled resin on the ceramic surface after the application of silane, the current literature[5] offers little information about luting purposes. It is likely that the use of an unfilled resin will improve bond strength and adaptation of substrates along the ceramic-resin cement interface, as observed by Naves and others.[5]

Therefore, the aim of this study was to evaluate the influence of various HF concentrations on the μ–shear bond strength (μSBS) of EST and EMX ceramics when using a resin cement, with or without the application of an unfilled resin after applying silane, while also evaluating the modes of failure.

Table 1: *Group Descriptions*

Group	Surface Treatment		
	Hydrofluoric Acid Concentration, %	Postetching Treatment	Ceramic
G1	1	Silane	IPS Empress Esthetic
G2	1	Silane + unfilled resin	
G3	2.5	Silane	
G4	2.5	Silane + unfilled resin	
G5	5	Silane	
G6	5	Silane + unfilled resin	
G7	7.5	Silane	
G8	7.5	Silane + unfilled resin	
G9	10	Silane	
G10	10	Silane + unfilled resin	
G11	15	Silane	
G12	15	Silane + unfilled resin	
G13	1	Silane	IPS e.max Press
G14	1	Silane + unfilled resin	
G15	2.5	Silane	
G16	2.5	Silane + unfilled resin	
G17	5	Silane	
G18	5	Silane + unfilled resin	
G19	7.5	Silane	
G20	7.5	Silane + unfilled resin	
G21	10	Silane	
G22	10	Silane + unfilled resin	
G23	15	Silane	
G24	15	Silane + unfilled resin	

This present study also characterized the morphology aspect of the etched surfaces and the interfaces created between the substrates. The hypotheses tested were as follows: 1) Different HF concentrations do affect the μSBS; and 2) The unfilled resin does influence the bond strength and interface homogeneity.

METHODS AND MATERIALS

Ceramic Blocks

One hundred forty-four square ceramic blocks (8 mm × 8 mm × 3.0 mm thick) were fabricated for each type of ceramic, EST and EMX, in accordance with the manufacturer's instructions. Square wax patterns were made, sprued, and invested with phosphate-based material (Esthetic Speed or IPS PressVest Speed, Ivoclar Vivadent), and the wax was eliminated in an automatic burn-out furnace (Vulcan A- 550, Degussa-Ney, Yucaipa, CA, USA) at 850°C for one hour. The EST and EMX ingots were pressed into the investment molds in an automatic press furnace (EP 600, Ivoclar Vivadent). After cooling, the specimens were divested, placed in a horizontal position, embedded in polyester resin (Resapol T208, Difibra/Fiberglass Ltda, Mogi das Cruzes, SP, Brazil) in rigid polyvinyl chloride tubes with a 20-mm diameter and 20-mm height, and submitted to wet polishing with 600-, 1200-, and 2000-grit silicon carbide abrasive papers (Norton SA, São Paulo, SP, Brazil) to obtain a flat surface.

Ceramic Surface Treatments

The ceramic blocks (144 in total) were randomly divided into 24 groups (n=6), as defined by the HF concentrations (1%, 2.5%, 5%, 7.5%, 10%, and 15%; Formula & Ação, São Paulo, SP, Brazil). Table 1 presents a description of the tested groups. In groups 1 through 12, the EST specimens were etched for 60 seconds and rinsed with distilled water for one minute. The specimens in groups 13 to 24, the EMX ceramic blocks were etched for 20 seconds and rinsed with distilled water for one minute. All specimens were then ultrasonically cleaned in distilled water for one minute and dried with compressed oil-free water/air spray. A silane coupling agent (RelyX Ceramic Primer, 3M ESPE, St Paul, MN, USA) was applied onto all ceramic surface specimens and allowed to air dry for 15 seconds, followed by air heat drying for 45 seconds. Half of the specimens from each group received a thin layer of unfilled resin (Scotchbond MultiPurpose, 3M ESPE, Seefeld, Germany) that was light activated for 10 seconds using a LED source (UltraLume 5, Ultradent Inc, South Jordan, UT, USA) with an irradiance of 1.100 mW/cm^2.

Bond Strength Testing

The method used to obtain specimens for the μSBS testing and the design of the test[5,25] are shown in Figure 1. Elastomer molds (Express STD, 3M ESPE), which were 3 mm thick and contained three cylinder-shaped orifices (1 mm in diameter), were placed onto the ceramic surfaces, allowing the delimitation of the bonding area. The orifices were filled with resin cement (Variolink II, shade A3; Ivoclar Vivadent), and a transparent polyester strip and glass plate were placed over the filled mold. A 250*g* cementation load was applied for two minutes. The glass plate was removed and the resin cement was light activated for 40 seconds using a LED source (UltraLume 5, Ultradent). The specimens were then stored in distilled water at 37°C for 24 hours. Three cylinders were built up on each ceramic block, with 18 cylinders tested for each group.

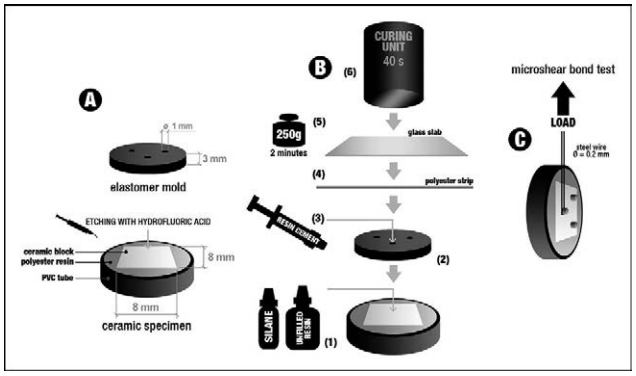

Figure 1. *Experimental setup of the study. After etching, (1) silane was applied to the ceramic surface, and for half of the specimens, an unfilled resin was applied after applying the silane; (2) an elastomer mold with cylinder-shaped orifices was positioned onto the surface and photo-activation of the unfilled resin was performed; (3) orifices were filled with resin cement; (4) a polyester strip and glass slab were placed over the filled mold; (5) cementation load applied for two minutes; (6) photo-activation of the resin cement was facilitated.*

After storage, all resin cement cylinders were checked using optical microscopy (Olympus Corp, Tokyo, Japan) at 40× magnification, and those with irregularities, bonding defects, or flaws were eliminated. For the μSBS testing, a thin steel wire (0.2 mm in diameter) was looped around each cylinder and aligned with the bonding interface (Figure 1). The bonding test was conducted using a mechanical testing machine (model 4411; Instron; Canton, MA, USA) and at a crosshead speed of 0.5 mm/min until failure. The fractured specimens were examined under optical microscopy (Olympus Corp) at 40× magnification. Failure modes were classified as follows: adhesive (mode 1); cohesive within ceramic (mode 2); cohesive within resin cement (mode 3); and mixed, involving resin cement, ceramic, and composite (mode 4).

Statistical Analysis

Values of μSBS were calculated and the data provided in megapascals. For each group, six specimens were tested, and the average value of the three resin cylinders was recorded as the bond strength for each specimen. μSBS data were submitted to three-way analysis of variance, and multiple comparisons were performed using the Tukey post hoc test ($p<0.05$).

Scanning Electron Microscopy (SEM) Evaluation

In order to observe the surface characteristics of the conditioned surfaces, etched specimens for each HF concentration were sputter coated with gold (Balzers-SCD 050, Balzers Union, Aktiengeselischaft, Fürstentun, Liechtenstein) for 180 seconds at 40 mA. The specimens were then mounted on coded brass stubs and examined using SEM (LEO 435 VP, Cambridge, UK), operated at 20 Kv, by a single operator. Samples were examined under magnifications varying from 2000× to 3000×. Additionally, the EST or EMX ceramic blocks were sectioned, etched, and silane coated, and the same kind of ceramics were bonded to each other using resin cement to observe the morphology at the bonding interfaces for each group evaluated. After storage for 24 hours, the specimens were sectioned perpendicular to the bonding interface and embedded in epoxy resin (Buehler, Lake Buff, IL, USA) so that the ceramic-cement interfaces could be viewed. The specimens were wet-polished with 400-, 600-, 1200-, and 2000-grit silicon carbide abrasive papers, followed by polishing with 3-, 1-, and 0.5-μm diamond compounds. After polishing and ultrasonic cleaning, the specimens were gold sputter-coated (Balzers-SCD 050, Balzers Union). The cross-section profiles were examined under SEM (LEO 435 VP), focusing on the depth of etching, micromechanical entanglement and integrity, homogeneity, and continuity along the bonding interface, similar to what has been previously described by Naves and others.[5] Samples were examined under magnifications varying from 2000× to 3000×.

RESULTS

Microshear Bond Strength (μSBS)

The mean values of μSBS are shown in Table 2. Ceramic vs unfilled resin ($p=0.367$), ceramic vs HF concentration ($p=0.100$), and unfilled resin vs HF concentration ($p=0.196$) values did not show significant interaction of factors. The triple interaction between factors was not significant ($p=0.565$). Significant differences for unfilled resin ($p<0.001$) and HF concentration ($p<0.001$) were detected. No difference was detected for the ceramics ($p=0.957$). When unfilled resin was used, the mean value of μSBS was significantly higher when compared to values for the specimens prepared without it ($p<0.05$). When the ceramic material was compared, no statistical difference was found between the EST and EMX ceramics ($p>0.05$). For the HF concentrations, 10% and 15% showed mean values of μSBS that were significantly higher when compared to the 1% and 2.5% values ($p<0.05$). The concentration of 7.5% was significantly higher when compared to that of 1% ($p<0.05$). No statistical differences were found among the other concentrations ($p>0.05$).

Table 2: *Means of Microshear Bond Strength ± Standard Deviation (MPa) for All Groups. Parenthetical Values under Tukey % Indicate the Overall Mean Bond Strength for the Indicated Hydrofluoric Acid Concentration.*

Ceramic	Hydrofluoric Acid Concentration, %	Unfilled Resin[a]		Tukey, %[a]
		With	Without	
IPS Empress Esthetic (29.8)[b]	1	27.6 ± 6.3	20.7 ± 5.3	
	2.5	27.3 ± 7.9	23.8 ± 5.7	
	5	32.5 ± 9.1	31.1 ± 5.9	
	7.5	32.1 ± 3.1	31.8 ± 6.1	1 (25.2) c
	10	39.2 ± 6.7	31.3 ± 5.5	2.5 (27.2) bc
	15	32.3 ± 3.2	27.6 ± 6.2	5 (30.1) abc
IPS e.max Press (29.9)[b]	1	29.2 ± 5.4	23.6 ± 5.6	7.5 (31.4) ab
	2.5	29.4 ± 9.8	28.3 ± 5.7	10 (33.3) a
	5	28.5 ± 3.9	28.1 ± 6.1	15 (31.8) a
	7.5	34.4 ± 3.3	27.3 ± 5.4	
	10	36.1 ± 4.1	26.6 ± 5.7	
	15	38.5 ± 6.1	28.9 ± 3.1	
Tukey		32.24 A	27.4 B	

[a] *Same capital letters indicate no significant differences with or without unfilled resin application ($p>0.05$).*
[b] *No significant differences for ceramic ($p>0.05$), and means followed by different lowercase letters indicate significant differences for hydrofluoric acid concentrations (%) ($p<0.05$).*

Failure Analysis

A descriptive analysis of failure modes is shown in Table 3. A predominance of cohesive within ceramic failure (mode 2) was found for the ceramic EST. For the EMX, a predominance of adhesive failures (mode 1) was detected for the 1% to 5% acid concentrations and failures that were cohesive within resin cement (mode 3) for the 7.5% to 15% acid concentrations.

SEM Evaluation

SEM images of etched surfaces with 1%, 2.5%, 5%, 7.5%, 10%, and 15% HF concentrations are shown in Figures 2 and 3. Figures 4 and 5 present images of the ceramic/resin cement bonding interfaces.

The EMX ceramic presented with greater vitreous phase dissolution and exposure of lithium disilicate crystals with increased HF concentrations. Figure 2A and B exhibited slight vitreous phase dissolution, and Figure 2C and D showed similar patterns, with more evident vitreous dissolution. The images in Figure 2E and F show greater vitreous phase dissolution on the ceramic surface due to the higher HF concentration. Figure 3 presents images of acid etching with the various HF concentrations on the EST ceramic surface. Minimal vitreous phase dissolution can be observed in Figure 3A, while Figure 3B presented slightly greater vitreous phase dissolution. The images in Figure 3C and D present even greater vitreous phase dissolution, causing microporosities. Figure 3F revealed the formation of fissures arising from surface etching.

Figure 4 represents the bonded interface for EMX. The images in Figure 4B, E, and F demonstrated the deficient quality of bonding between ceramic and resin cement with unfilled voids (indicated with a white arrow). When the unfilled resin was used, as in the images in Figure 4A, C, and D, a completely infiltrated interface is seen between ceramic, unfilled resin, and resin cement. The same situation is shown for EST (Figure 5).

DISCUSSION

Alterations in the morphology of the ceramic surface may promote a better bond strength.[26] HF is a modifier and etching agent indicated for ceramic that contains silica,[14,27] acting to dissolve the vitreous phase, exposing crystals and resulting in microporosities on the ceramic structure.[27-30] This provides increased surface area and improved bonding quality[6,11,17,27,28,30,31] and promotes better contact between the restoration material and the resin cement.[4,13,14,28,32]

In this study, the first hypothesis, which stated that different HF concentrations applied on ceramic surface would affect the μSBS between ceramic and resin cement, was accepted. The mean results showed that lower values for μSBS were obtained for HF concentrations of 1% and 2.5% for both EST and EMX, with a statistically significant difference when compared to the values associated with HF concentrations of 10% and 15%. Lower concentrations were not enough to properly dissolve the

Table 3: *Failure Mode Analysis of the Debonded Specimens (%) Among Groups*[a]

Groups	Failure Modes			
	Mode 1	Mode 2	Mode 3	Mode 4
IPS Empress Esthetic (EST)				
1 – EST1	44	56	0	0
2 – EST1 *UR*	50	50	0	0
3 – EST 2.5	28	66	6	0
4 – EST 2.5 *UR*	28	66	0	6
5 – EST 5	0	33	56	11
6 – EST 5 *UR*	6	88	0	6
7 – EST 7.5	0	56	28	16
8 – EST 7.5 *UR*	0	88	6	6
9 – EST 10	0	78	11	11
10 – EST 10 *UR*	11	73	0	16
11 – EST 15	6	94	0	0
12 – EST 15 *UR*	6	61	11	22
IPS e.max Press (EMX)				
13 – EMX 1	100	0	0	0
14 – EMX 1 *UR*	100	0	0	0
15 – EMX 2.5	88	6	6	0
16 – EMX 2.5 *UR*	88	6	6	0
17 – EMX 5	50	0	22	28
18 – EMX 5 *UR*	88	0	6	6
19 – EMX 7.5	33	0	56	11
20 – EMX 7.5 *UR*	44	0	56	0
21 – EMX 10	6	6	44	44
22 – EMX 10 *UR*	11	6	61	22
23 – EMX 15	0	6	50	44
24 – EMX 15 *UR*	28	6	44	22

Abbreviation: UR, unfilled resin.
[a] *Failure modes were classified as follows: adhesive (mode 1); cohesive within ceramic (mode 2); cohesive within resin cement (mode 3); and mixed, involving resin cement, ceramic, and composite (mode 4).*

vitreous phase and exhibited minimal vitreous phase dissolution, in contrast to higher concentrations (Figures 2 and 3). This is likely explained by the presence of fewer microporosities, promoting reduced contact between the ceramic surface and resin cement, resulting in less mechanical interlocking and lower bond strengths, as the shear bond strength is directly influenced by ceramic surface roughness.[4] The degree of ceramic dissolution is proportional to the HF concentration (Figures 2 and 3) and may promote higher values of bond strength.[32] It has been shown that bond strength is more directly influenced by the type of etching agent than it is by the resin cement.[33]

When the ceramics were etched with 7.5% HF, the mean value of μSBS was statistically significantly higher when compared to the values associated with the HF concentration of 1%, although the value associated with the 7.5% concentration was not statistically different from those of the other concentrations. The 7.5% concentration promoted effective dissolution of the vitreous phase in both ceramics. This is likely because 7.5% HF was able to promote sufficient change to the ceramic surface, which improved the mechanical interlocking of the resin cement to the ceramic structure. Therefore, the 7.5% concentration could be as easily indicated for clinical use as the widely used 10%. HF can be harmful and particularly aggressive to soft tissues, but symptoms may not be apparent immediately after exposure because the lesion severity is directly related to the exposure time and the acid concentration.[15] Even though HF is not applied on soft tissue, less concentrated HF would cause less injury in accidental contact situations.

Chen and others[7] and Zogheib and others[34] found rougher feldspathic ceramic surfaces with increased

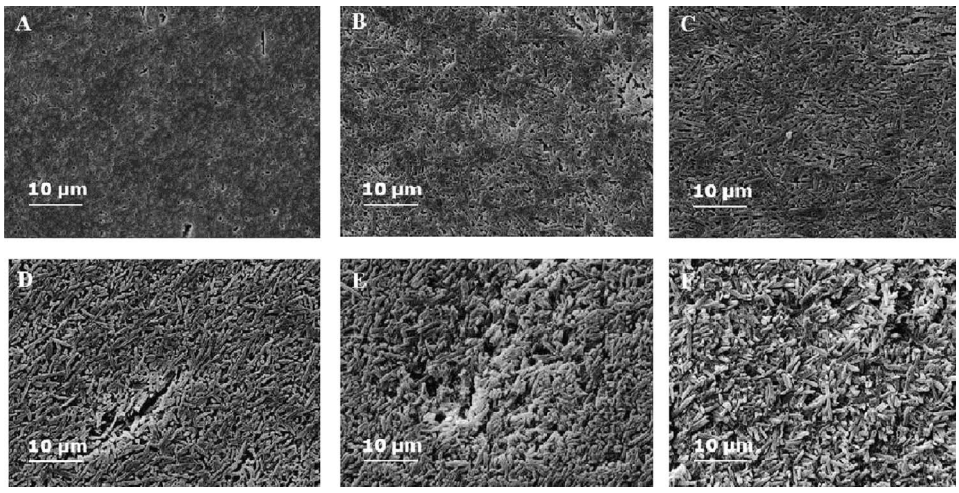

Figure 2. *Images resulting from acid etching with hydrofluoric acid (HF) on ceramic surface (IPS e.max Press). For HF concentrations (A: HF 1%, B: HF 2.5%; C: HF 5%; D: HF 7.5%; E: HF 10%; and F: HF 15%), different etching patterns were found with distinct degrees of vitreous phase dissolution and exposure of lithium disilicate crystals. Images A and B show poor dissolution of the vitreous phase, while there is an increase in the degree of vitreous phase dissolution with higher HF concentrations.*

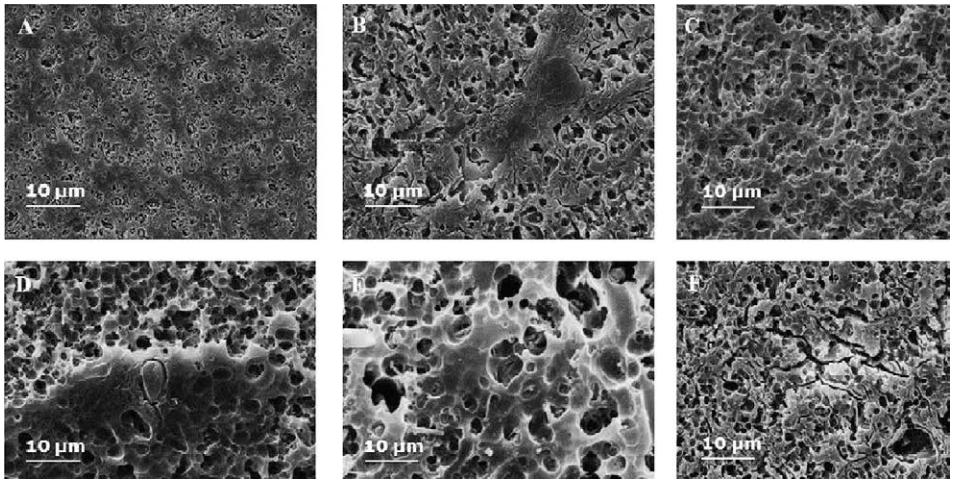

Figure 3. *Images resulting from acid etching with hydrofluoric acid (HF) on ceramic surface (IPS Empress Esthetic). For HF concentrations (A: HF 1%, B: HF 2.5%; C: HF 5%; D: HF 7.5%; E: HF 10%; and F: HF 15%), different etching patterns were found with distinct degrees of vitreous phase dissolution. Image A represents a poor dissolution of vitreous phase, while there is an increase in the degree of vitreous phase dissolution with higher HF concentrations.*

etching times (ranging from 20 to 180 seconds). Thus, if a greater etching time were adopted with the 1% and 2.5% HF used in this current research, it is likely that improved bond strengths could be obtained, providing safer patterns for working with HF. However, if the etching time had been increased, the resulting stronger and deeper ceramic degradation could have weakened its structure.[34,35] Beyond that, the buffering-acid process can increase the pH, as the reactants of the reaction are consumed, decreasing its etching effect. Therefore, dentists should be extremely careful when using an increased etching time with HF, considering that this protocol would not necessarily result in a better etched surface and/or bond strength.

It has been shown[5] that the effectiveness of bonding using only silane depends on the ability of the bonding agent to fill the irregularities and to provide a close contact between the resin cement and the ceramic. However, when an unfilled resin was used, it infiltrated the etched surface irregularities and improved the adaptation of the resin cement/ceramic interface and bond strength. In this present study, when an unfilled resin was used with a silane coupling agent for both ceramics, the mean values of μSBS were significantly higher when compared to those of specimens that received only silane. Therefore, the second hypothesis was also accepted. Similar results were found by Hooshmand and others[22] and Naves and others,[5] who observed greater means of μSBS when an unfilled resin was applied after the application of a silane. Significant evidence was also found when the unfilled resin was applied to the bonded interface and evaluated under SEM (Figures 4A,C,D and 5C,D,E), in which complete penetration of unfilled resin in ceramic irregularities and a homogeneous interface were found between the ceramic and resin cement. This occurred as a result of the lower viscosity presented by the unfilled resin in relation to the resin cement[5] and because of a better interaction of the unfilled resin when applied to the ceramic surface treated

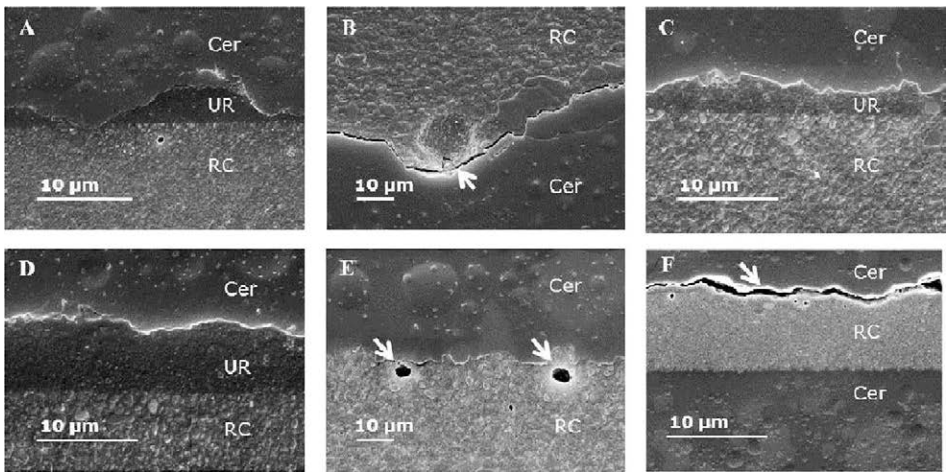

Figure 4. *Images resulting from bond interface analysis (IPS e.max Press). RC, resin cement; Cer, ceramic; UR, unfilled resin; HF, hydrofluoric acid. (A) HF 1%; (B) HF 2.5%; (C) HF 5%; (D) HF 7.5%; (E) HF 10%; (F) HF 15%. Image A shows an interaction without failures among the ceramic, unfilled resin, and resin cement. The white arrow in image B indicates an incomplete interaction between ceramic and resin cement. The unfilled resin was able to penetrate the ceramic in images C and D. The white arrows in images E and F indicate failures at the resin cement-ceramic interface, when the unfilled resin was not applied.*

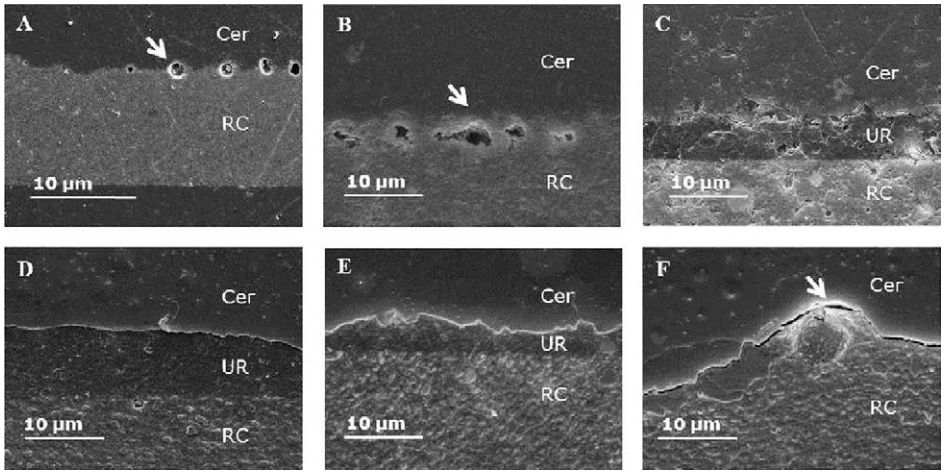

Figure 5. *Images resulting from bond interface analysis (IPS Empress Esthetic). RC, resin cement; Cer, ceramic; UR, unfilled resin; HF, hydrofluoric acid. (A) HF 1%; (B) HF 2.5%; (C) HF 5%; (D) HF 7.5%; (E) HF 10%; (F) HF 15%. Images A and B show incomplete resin cement penetration of surface without the application of the unfilled resin (white arrow). Images C, D, and E show complete penetration of the unfilled resin into ceramic irregularities. However, image F shows an incomplete interaction between the resin cement and ceramic (white arrow).*

with silane.[6,17,23] Under these conditions, the unfilled resin more easily penetrates the etched ceramic surface, when compared to the resin cement, which has filler particles in its composition that hamper its penetration into deeper irregularities on the ceramic surface. According to Naves and others,[5] the unfilled resin fills voids that the resin cement cannot. On the ceramic surfaces that did not receive the application of the unfilled resin, the interface images suggest voids unfilled by the resin cement, causing a nonhomogeneous interface (Figures 4B,E,F and 5A,B,F). This could lead to stress concentration and induce clinical failure since the bonding interface plays an important role in the long-term durability of ceramic restorations.[3] This situation may also have negatively affected the immediate bond strengths.

The analysis of failure modes showed a predominance of cohesive failures within ceramic for the EST ceramic. The EMX presented a predominance of adhesive failures for the 1% to 5% HF concentrations, indicating poor bond quality, and cohesive within–resin cement failures for the 7.5% to 15% HF concentrations (Table 3), most likely due to improved micromechanical interaction between the ceramic and resin cement.[5] This difference can be explained by the low resistance to crack propagation in the EST ceramic due to dissimilar ceramic composition, nonhomogeneous stress distribution at the interface (as produced by the microshear bond test),[36] or the influence of greater HF concentrations on the leucite-based ceramic.

The EST ceramic is a glass-based ceramic reinforced by leucite crystals, which is about 35.5% ± 5% vol and is indicated for inlays, onlays, crowns, and for veneering other core-ceramics. EMX is a lithium disilicate–reinforced glass ceramic, containing about 70% ± 5% vol of crystalline phase. These features provide improved mechanical properties for the EMX;[1] therefore, it is indicated for three-unit fixed partial dentures up to the second premolar.[37] According to de Melo and others,[38] the higher content of the crystalline phase and lower vitreous phase causes fewer cohesive failures in the ceramic. The fact that the EST is submitted to higher etching times compared to EMX might also explain the cohesive ceramic failures found in this current research. Therefore, the bond quality should not only be evaluated by bond strength values but also by its association with failure analysis and fractography to provide a better clinical preview of the performance.[22,36]

The data and images in the present study demonstrate that the HF concentration influenced the μSBS and influenced the surface and ceramic/resin cement bonding interface. Moreover, the application of a low-viscosity unfilled resin after application of a silane may better infiltrate the etched surface of the ceramic, increasing the bond strength, the adaptation of the interface resin cement-ceramic, and possibly its clinical longevity. Therefore, the use of an unfilled resin should be encouraged in clinical practice. However, the thickness of this layer should be as thin as possible. Care should be taken in clinical practice, regardless of the HF concentration used, because HF is toxic and capable of causing severe trauma to soft tissues. Therefore, the etching procedure must be done with personal protective equipment in well-ventilated rooms to avoid any further damage to the professionals. Future studies should be carried out using different cementation loads, viscosities of the resin

cement, thermal cycling, and degradation of the unfilled resin.

CONCLUSIONS

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

1. The various HF concentrations influenced the bond strength and surface/interface morphology.
2. Application of an unfilled resin increased the μSBS; it also promoted better infiltration of the irregularities of the etched surfaces for both ceramics.
3. No statistical difference was found in μSBS between the two ceramics. The EST showed a tendency for cohesive failure in ceramic, and EMX presented with adhesive and cohesive failures in resin cement.

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Conflict of Interest

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

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Effect of Thermocycling, Degree of Conversion, and Cavity Configuration on the Bonding Effectiveness of All-in-One Adhesives

HM El-Damanhoury • M Gaintantzopoulou

Clinical Relevance

The poor results of some all-in-one dentin adhesives tested may indicate that clinicians should be cautious with selection of this class of materials for high-C-factor or deep-class II applications.

SUMMARY

The aim of this study was to compare five all-in-one bonding agents with respect to microleakage, microtensile bond strength (μTBS), degree of conversion (DC) and the impact of cavity configuration. The materials tested were Adper Easy Bond, Clearfil S3 Bond, iBond, Optibond All-in-One, Xeno IV, and Adper Single Bond Plus as a control. The DC of each adhesive was measured on the surfaces of dentin discs (n=5) by attenuated total reflectance Fourier transform infrared spectroscopy. One hundred and forty-four extracted human molars were randomly divided and assigned to one of the five tested adhesives and the control group. The μTBS to dentin was measured on flat occlusal dentin with and without thermocycling and to the gingival floor dentin of class II cavities (n=8). All specimens were restored with Filtek Z250 resin composite. Class II samples were immersed in a 5% methylene blue dye solution for 24 hours, and microleakage was examined under a stereomicroscope. Micromorphological analysis of demineralized/deproteinized specimens was done using scanning electron microscopy. The DC and microleakage data were statistically analyzed by one-way analysis of variance (ANOVA) and μTBS data by two-way ANOVA followed by a Bonferroni multiple comparison *post hoc* test (α=0.05) and Weibull-distribution survival analysis. The relation between differ-

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ent variables and μTBS and microleakage was tested by the Pearson correlation coefficient and regression statistics. A moderate direct relation between DC and μTBS durability was found for all the adhesives tested. Significant wide variations exist among the results obtained for single-bottle adhesives tested regarding their μTBS and microleakage. Some of the all-in-one materials tested have shown significantly inferior results under a high C-factor or after aging. The use of these materials should be carefully considered.

INTRODUCTION

In the past few decades, there has been a significant increase in patient demand for esthetic restorations, and in response, direct resin composites have been widely used not only in the anterior area but also in various cavities in the posterior teeth. The major shortcoming of those adhesive restorations is their limited durability *in vivo*.[1] A weakened bond, poor marginal adaptation, and subsequent gap formation at the resin-dentin interface are common with these restorations, which may be followed by failure of the restoration in the form of microleakage, hypersensitivity, and recurrent caries.[2]

Establishing adequate bonding to dentin has proven to be a challenging task. Self-etch adhesives were introduced to overcome the technique sensitivity that might be encountered with the etch-and-rinse systems. These systems incorporate acidic components to partially demineralize and infiltrate dentin simultaneously.[3] Two-step self-etch adhesives show good and durable bonding to dental tissues.[4] On the contrary, various studies report conflicting results on the performance of one-step self-etch (all-in-one) adhesives.[5] Although excellent immediate and short-term bonding effectiveness of this class of dental adhesives has been revealed, the durability and stability of the bonded interfaces on dentin of some all-in-one bonding systems remain questionable.[6] The poor performance was attributed mainly to the hydrophilic nature of these materials and their liability to degradation by water or enzymatic activity of matrix metalloproteinases in the long term.[7] Optimal infiltration of the adhesive into the demineralized substrate and a high degree of conversion (DC) are essential in establishing long-lasting bonds.[8] Moreover, polymerization contraction exposes restoration-tooth interfaces to tensile stresses, leading to interface degradation and decreases in microtensile bond strength (μTBS), especially when restoring cavities with a high C-factor.[9,10]

The purpose of the present study was to 1) compare bond strength and microleakage of different all-in-one adhesives, 2) determine the effect of aging by thermocycling and cavity configuration on the bonding effectiveness, and 3) determine the relation between degree of conversion (DC), cavity configuration, microleakage, and bond strength. The null hypotheses tested in this investigation were that there is no significant difference among products tested and that the bond strength and microleakage of those adhesives are not a function of aging, degree of adhesive cure, or cavity configuration.

METHODS AND MATERIALS

Five all-in-one adhesives and one etch-and-rinse adhesive as the control group were evaluated in this study. Names and compositions of the tested materials are listed in Table 1, and their application procedures are listed in Table 2.

Degree of Conversion

Thirty 1.0-mm-thick dentin discs were prepared from extracted sound human molars. Teeth were cleaned from stains, calculus, and soft tissues with an ultrasonic scaler and stored in 0.5% chloramine-T at 4°C and used within 1 month. Dentin disc surfaces were polished with SiC papers up to 1000-grit under running water for 1 minute to create a standardized smear layer. Dentin discs were divided equally into six groups (n=5), and each group was assigned to one of the five all-in-one adhesives or control. The tested adhesives were placed on dentin and light cured according to the manufacturer's instructions (Table 2) with an LED light-curing unit (Demetron A.1, Kerr/Sybron, Orange, CA, USA) with a curing distance of 0.5 mm operating at 1600 ± 10 mW/cm^2. The light intensity was verified every five specimens using a digital curing radiometer (Cure Rite, Dentsply Caulk, Milford, DE, USA). Ten minutes after exposure to the curing light, the cured films were rinsed with acetone to remove the oxygen-inhibited layers and air-dried, and the DC was measured and analyzed by attenuated total reflectance Fourier transform infrared spectroscopy (FTIR; Spectrum GX, PerkinElmer, Coventry, UK). Spectra of the set materials were taken (400-4000-cm^{-1} wave number range, 4-cm^{-1} resolution, 40 scans coaddition, and 2.5-μm depth of analysis at 1000 cm^{-1}). Spectra were acquired for each material before polymerization to serve as a reference. The DC of each specimen was estimated on a relative

Table 1: *Names, Codes, Manufacturers, and Composition of Tested Adhesives*

Adhesive	Manufacturer	Composition
Adper Easy Bond (AP)	3M ESPE, St Paul, MN, USA	HEMA, bis-GMA, methacrylated phosphoric esters, 1,6-hexanediol dimethacrylate, methacrylate-functionalized polyalkenoic acid, silica filler (7 nm), ethanol, water, camphorquinone, stabilizers
Clearfil S3 (S3)	Kuraray America, New York, NY, USA	MDP, HEMA, bis-GMA, hydrophobic dimethacrylate, ethyl alcohol, water, photoinitiator, colloidal silica
iBond (IB)	Heraeus Kulzer, Hanau, Germany	UDMA, 4-MET, glutaraldehyde, acetone, water, photoinitiators, stabilizer
Optibond All-in-One (OB)	Kerr Corp, Orange, CA, USA	GPDM, mono- and difunctional methacrylate monomers, water, acetone, ethyl alcohol, camphorquinone, nanosilica and sodium hexafluorosilicate fillers
Xeno IV (XB)	Dentsply Caulk, Milford, DE, USA	Mono-, di-, and trimethacrylate resins, PENTA, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone, water
Adper Single Bond Plus (SB)	3M ESPE	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water, ethanol, silica nanofillers

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol A and glycidyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UDMA, urethane dimethacrylate; 4-MET, 4-methacryloxyethyltrimellitate; GPDM, glycerophosphate dimethacrylate; PENTA, dipentaerythritol pentaacrylate phosphate.

percentage basis with the two-band method and the tangent baseline technique.[11] The peaks of the aliphatic (C=C) bonds stretching vibrations at 1636 cm^{-1} were chosen as the analytical band, whereas the peaks of the aromatic (C=C) bonds stretching vibrations at 1607 cm^{-1}, which are not affected by the polymerization reaction, were selected as the reference band. The DC was calculated using methods commonly found in the literature[12,13] and according to the equation

$$\%DC = 1 - \frac{(AliphaticC=C/AromaticC=C)Polymer}{(AliphaticC=C/AromaticC=C)Monomer} \times 100$$

Table 2: *Application Procedures of Tested Adhesives*

Adhesive	Application Procedure
Adper Easy Bond (AP)	1. Apply adhesive to enamel and dentin (two layers) and scrub for 20 s 2. Gentle air blow for 5 s 3. Light cure for 10 s
Clearfil S3 (S3)	1. Adhesive application (two layers) 2. High-pressure airstream for 5 s 3. Light polymerize for 10 s
iBond (IB)	1. Application of adhesive (two layers) for 20 s with agitation 2. Start with gentle air blow, followed by a strong air blow for at least 5 s 3. Light cure for 20 s
Optibond All-in-One (OB)	1. Adhesive application and scrubbing for 20s (two layers) 2. Gentle air blow, then medium-force air dry for 5 s 3. Light cure for 10 s
Xeno IV (XB)	1. Adhesive application and scrubbed for 20 s (two layers) 2. Gentle air blow for 5 s 3. Light cure for 10 s
Adper Single Bond Plus (SB)	1. Etch enamel and dentin, with 37% phosphoric acid, rinse with water, and blot dry 2. Adhesive applications (two to three layers) and scrub for 15 s 3. Air blow for 5 s 4. Light cure for 10 s

Microtensile Bond Strength

One hundred and forty-four noncarious, nonrestored human molars were collected and treated as mentioned previously. Occlusal enamel was removed, and the exposed dentin on the occlusal surfaces was examined under a light microscope (Nikon Measurescope UM-2, Nikon Corp, Kanagawa, Japan) for any remnants of enamel or pulp exposure. A second cut was made in the root approximately 3 mm from the cemento-enamel junction, creating two parallel surfaces. All specimens were mounted on plastic blocks.

Bond Strength to Occlusal Flat Dentin Surfaces

Ninety-six of the prepared specimens were selected randomly, and an automatic grinder/polisher with a 600-grit SiC paper disc running at 120 rpm under water cooling for 1 min (Buehler, Lake Bluff, IL, USA) was used to create a standardized smear layer on the flat occlusal dentin. Specimens were randomly divided into six experimental groups. Each group was assigned to one of the five all-in-one adhesive systems and the control. Adhesive systems were applied according to the manufacturer's instructions. Specimens were restored with hybrid resin composite (Filtek Z250, 3M ESPE, St Paul, MN, USA) using two increments each 1.5 mm thick,

packed with the help of a plastic instrument, and cured for 20 seconds. Specimens were stored in double-distilled water at 37°C for 1 week to allow for bonded interface maturation before testing. Specimens of each group were divided in two equal subgroups (n=8). The first subgroup (flat TC) was subjected to 5000 thermal cycles between two water baths of 5°C and 55°C with a dwell time of 30 seconds at each temperature extreme (Thermocycler, Willytec, Munich, Germany) before μTBS testing. The second subgroup was tested right after water storage (flat non-TC).

Specimens were sectioned serially in a mesiodistal direction and perpendicular to the bonded surfaces, rotated 90 degrees and sectioned in the bucco-lingual direction to obtain 16 beams from each tooth ($0.8\pm0.2 \times 0.8\pm0.2$ mm). The four sticks taken from the center of the restorations were selected for μTBS testing to exclude the variable of degree of approximation of the beam to the outer enamel layer. Specimens were mounted on a μTBS tester (Bisco Dental Products, Schaumburg, IL, USA) with cyanoacrylate and stressed to failure. The maximum kilograms force necessary to break the bonds in tension was recorded. The bonded surface area at the adhesive interface was calculated at the fracture site using a digital micrometer with 0.01 mm precision. Bond strength was obtained and expressed in MPa by dividing the measured force by the cross-sectional area of the bonded surfaces in centimeters.

Bond Strength to Proximal Class II Cavities

The remaining 48 teeth were sectioned parallel to the long axis of the tooth to remove proximal enamel and expose superficial dentin at the two proximal surfaces of each tooth. An occlusal slot cavity was prepared at the mesial site of the tooth with standardized dimensions of 2.5 ± 0.25 mm facio-lingually, 1.5 ± 0.25 mesio-distally, and 3.0 ± 0.25 mm occluso-gingivally, using a high-speed carbide bur (FG 245, KG Sorensen, SP, Brasil) under constant water cooling. The bur was replaced after every two preparations. Teeth were randomly divided into six experimental groups (n=8). Each group was assigned to one of the five all-in-one adhesive systems or the control adhesive.

Adhesive systems were applied according to manufacturer's instructions, then a clear plastic strip (Mylar Matrix Strips, Patterson Dental Supply, St Paul, MN, USA) was applied around the crown of the tooth to cover the proximal surface and held in place with a metal paper clip applied on the buccal and lingual surfaces of the specimen. All cavities were restored with hybrid resin composite (Filtek Z250, 3M ESPE), using two increments, each 1.5 mm thick, and cured from the occlusal direction with an LED light-curing unit with the same curing protocol mentioned previously and subjected to thermocycling.

The apices of the roots were sealed with resin-modified glass ionomer restorative (Fuji II LC, GC America Inc, Alsip, IL, USA). The entire surface of each specimen was then covered with two coats of varnish up to 1-mm from the restoration margins. Specimens were soaked in an aqueous solution of 5% methylene blue dye for 24 hours at 37°C. Following dye exposure, the specimens were rinsed thoroughly with double-distilled water for 30 seconds and kept moist for bond strength testing.

Specimens were serially sectioned in a mesiodistal direction, starting from the tooth restoration interface perpendicular to the gingival floor, into four slices, each 0.8 ± 0.1 mm in thickness. The outer two slices were separated for microleakage testing and micromorphological analysis, and the central two slices were rotated 90 degrees and sectioned again to obtain two beams with a cross-sectional area of 0.8×0.8 mm. The μTBS was tested and calculated as for the flat-surface specimens.

The debonded microtensile specimens were dehydrated in a desiccator containing dehydrated silica gel at room temperature for 24 hours, mounted on aluminum stubs, sputter coated with 100 Å gold-palladium, and examined to identify the failure mode by low-vacuum scanning electron microscopy (SEM; JSM 5310LV, JEOL Inc, Tokyo, Japan) running with a working distance of 20 mm at 10 kV of accelerating voltage and 60 mA of probe current. Micrographs were collected at magnifications up to 500×. The failures were characterized as adhesive when failure occurred either between adhesive resin and dentin or between adhesive resin and composite, cohesive when failure occurred within the adhesive layer or composite, and mixed when including two different types of failures.

Microleakage Test

The two outer slabs from each tooth were polished with SiC papers of increasing fineness (600-1200 grit) to create uniform flat surfaces and ultrasonically cleaned in distilled water for 10 minutes to remove any superficial debris created during the cutting and polishing procedures. Slabs were evaluated under a digital multiaxis dimensional measurement device (Quadra-chek 200, Metronics Inc,

Table 3: *Weibull Parameters and Mean Microtensile Bond Strength (μ-TBS) to Flat Surfaces With (TC) and Without Thermocycling (Non-TC) and to Class II Gingival Floor Dentin, Degree of Conversion (DC), and Microleakage of Adper Easy Bond (AP), Clearfil S3 Bond (S3), ibond (IB), Optibond All-in-One (OB), Xeno IV (XB), and Adper Single Bond Plus (SB)*

Material	μTBS to Flat Surface		μTBS to Class II Gingival Floor			DC %	Microleakage (Dye Penetration) (mm)
	Flat Non-TC (MPa)	Flat TC (MPa)	Class II TC (MPa)	Weibull Characteristic Strength (MPa)	Weibull Modulus		
AP	43.55 (6.2)^{Ba}	36.64 (6.6)^{ABb}	3.5 (1.0)^{Cc}	4.0	7.6	82.56 (1.88)^{AB}	2.9 (0.2)^{A}
S3	39.62 (7.1)^{Ba}	33.79 (3.8)^{Ba}	23.7 (11.2)^{ABc}	20.0	7.4	84.76 (3.20)^{A}	1.6 (0.2)^{CD}
IB	26.75 (4.2)^{Ca}	21.12 (4.5)^{Ca}	12.4 (9.3)^{DEc}	12.0	2.6	62.15 (3.36)^{D}	2.3 (0.2)^{B}
OB	48.40 (8.0)^{Ba}	33.32 (7.2)^{Bb}	18.8 (7.1)^{BDc}	18.0	9.0	63.50 (2.08)^{D}	1.2 (0.1)^{D}
XB	25.88 (5.5)^{Ca}	20.17 (3.8)^{Ca}	4.9 (2.4)^{CEc}	7.0	7.4	70.99 (3.74)^{C}	1.9 (0.2)^{C}
SB	58.17 (10.1)^{Aa}	43.56 (8.4)^{Ab}	23.9 (6.2)^{Ac}	27.0	7.3	77.02 (2.34)^{B}	1.4 (0.1)^{CD}

** Within a row, same lowercase superscript letters show mean values with no statistically significant difference (p>0.05).*
*** Within a column, same uppercase superscript letters show mean values with no statistically significant difference (p>0.05).*

Bedford, NH, USA) connected to a Measurescope (UM-2, Nikon) to measure the total depth of dye penetration in multiple axes. Dye penetration at the tooth-restoration interface at the gingival margin and axial wall of each slab was recorded in millimeters, and mean dye penetration of each tooth was calculated from the average of the readings of the two outer slabs.

Micromorphological Analysis

After the microleakage test, one of the outer slabs was selected randomly from each tooth and immersed in 6 N hydrochloric acid for 1 minute to demineralize the dentin, rinsed with tap water for 5 minutes, and then deproteinized by immersion in 2.5% sodium hypochlorite for 5 minutes and rinsed again with tap water for 5 minutes. Slabs were desiccated, sputter coated, and examined under SEM for morphological analysis of the bonded interface. Micrographs were collected at different magnifications up to 5000×.

Statistical Analysis

The μTBS results were analyzed with statistical software (SPSS version 20.0, SPSS Inc, Chicago IL, USA) using two-way analysis of variance (ANOVA) and a Bonferroni *post hoc* multiple comparison test (α=0.05). To estimate bonding performance, the μTBS data were also analyzed using Weibull-distribution survival analysis; the analysis included a frailty term to correlate the measurements from beams coming from the same specimen, following the protocol recommended by Eckert and Platt in 2007.[14] Specimens that spontaneously debonded were treated as left-censored at the lowest measured strength, and specimens that debonded due to cyanoacrylate failure or that did not fail prior to the end of testing were treated as right-censored at the highest measured strength. If the failure of these specimens was at a lower value, then they were treated as censored at the measured MPa. The DC and microleakage results were analyzed using one-way ANOVA followed by a Bonferroni *post hoc* multiple comparison test (α=0.05). Correlation between DC, microleakage, μTBS, thickness of the hybrid layer, and resin tag length was analyzed by calculating the Pearson correlation coefficient and regression statistics.

RESULTS

Mean values, standard deviations of DC, μ-TBS, and microleakage (dye penetration) of the five all-in-one adhesives tested and the control adhesive material in all experimental conditions are presented in Tables 3 and 4. The Weibull-distribution curve is presented in Figure 1. Typical failure patterns of the six adhesives tested are listed in Table 4 and illustrated in Figure 2a through f, and micromorphological analysis of the adhesive-dentin interfaces is presented in Figure 3a through f.

Degree of Conversion

According to the results of the present study, Clearfil S3 Bond (S3) showed the highest values (84.76±3.20) of DC; however, there was no statistically significant difference between S3 and Adper Easy Bond (AP; 82.56±1.88). Self-etch ibond (IB) and Optibond (OB) adhesives showed the lowest DC of all products tested.

Table 4: *Mode of Failure Percentage (%) of Adper Easy Bond (AP), Clearfil S3 Bond (S3), ibond (IB), Optibond All-in-One (OB), Xeno IV (XB), and Adper Single Bond Plus (SB)*

Material	Mode of Failure (%)		
	Adhesive	Cohesive	Mixed
AP	16	—	84
S3		—	100
IB	96	—	4
OB	66	13	31
XB	31	—	69
SB	79	—	21

** Adhesive failures are either between adhesive resin and dentin or between adhesive resin and composite. Cohesive failures are within adhesive layer or composite. Mixed failures includes two different types of failures.*

Microtensile Bond Strength

According to the results, when bonded to flat surfaces (flat non-TC), the control group Adper Single Bond Plus (SB) showed the highest bond strength (58.17±10.1) among all adhesives tested, while the lowest values were recorded for IB (26.75±4.2) and Xeno IV (25.88±5.5). When specimens were subjected to thermocycling (flat TC), all bond strength values were negatively affected; however, this reduction was statistically significant for SB, OB, and AP only. There was a moderate correlation between DC and μTBS reduction after thermocycling (Pearson coefficient=0.5116).

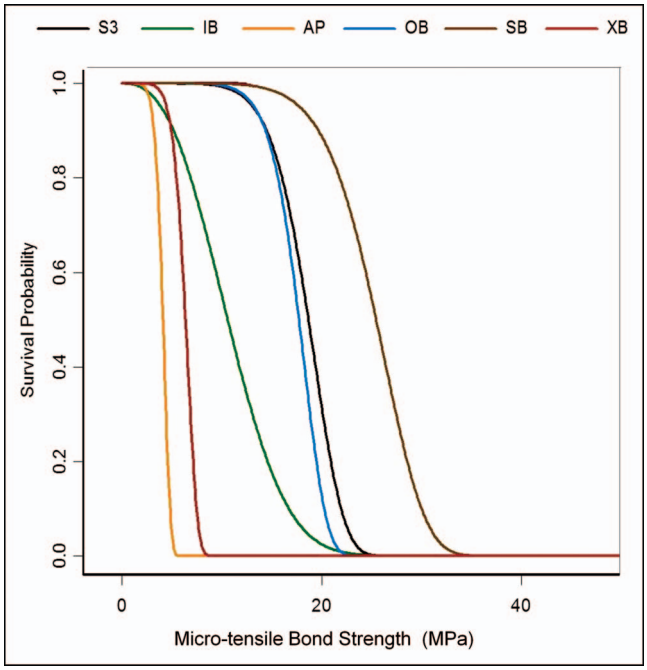

Figure 1. *Weibull-estimated survival probability of microtensile bond strengths (μ-TBS) to class II gingival floor dentin of Adper Easy Bond (AP), Clearfil S3 Bond (S3), ibond (IB), Optibond All-in-One (OB), Xeno IV (XB), and Adper Single Bond Plus (SB).*

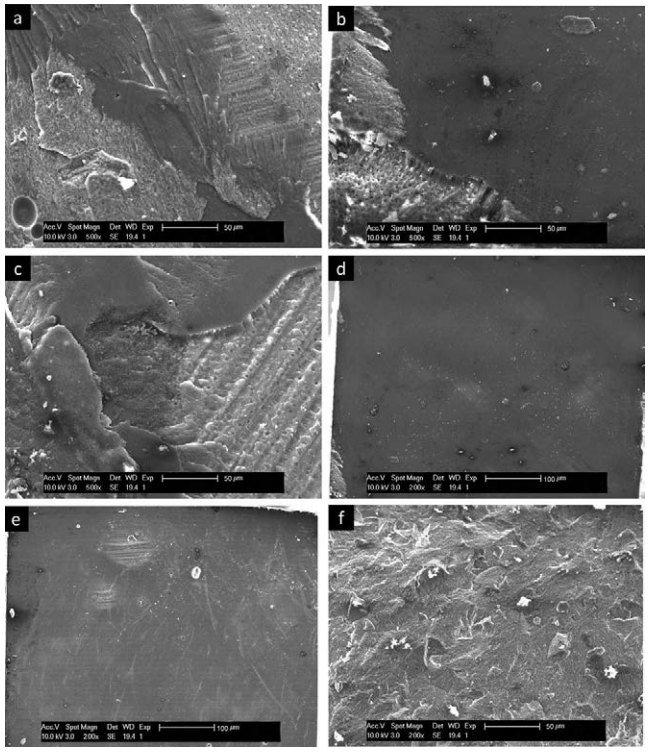

Figure 2. *Representative examples of failure mode as seen by scanning electron microscopy (SEM) on the bottom dentin side (class II restorations). (a): Optibond All-in-One (OB). (b): Adper Easy bond (AP). (c): Clearfil S3 Bond (S3). (d): ibond (IB). (e): Xeno IV (XE). (f) Adper Single Bond Plus (SB).*

Comparing μTBS to the gingival floor of the class II cavity (class II TC) with that of flat surfaces after thermocycling (flat TC), all bond strength values for class II TC were significantly lower than those for the flat TC group for all adhesives tested. Control group SB and S3 showed the highest μTBS (23.9±6.2 and 23.7±11.2, respectively), while μTBS values reported for AP (3.5±1.0) and XB (4.9±2.4) were very low, with no statistically significant difference. High standard deviations, a low Weibull modulus, and a high number of pretesting failures were recorded for both products.

Regarding failure patterns (Table 4), in flat dentin surfaces, most of the failures were recorded as mixed adhesive and/or cohesive regardless of adhesive. However, in the class II cavity dentin gingival floor (class II TC), only S3 showed 100% mixed-type failure (Figure 2c). The rest of the adhesives showed both mixed and adhesive failures with the IB and control group SB failing adhesively at a high percentage (96% and 79%, respectively).

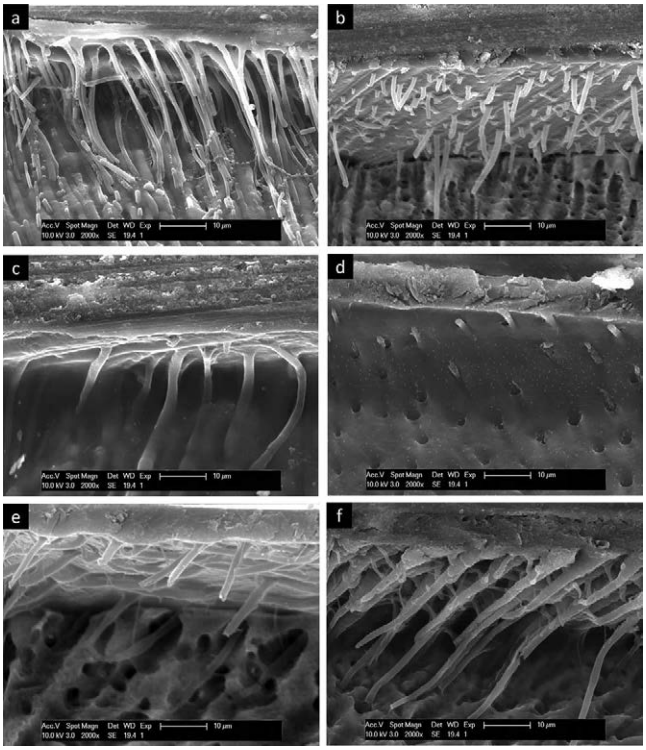

Figure 3. *Representative examples of bonded interface analysis of demineralized/deproteinized samples (class II bottom dentin) seen by scanning electron microscopy (SEM). (a): Optibond All-in-One (OB). (b): Adper Easy Bond (AP). (c): Clearfil S3 Bond (S3). (d) ibond (IB). (e): Xeno IV (XE). (f): Adper Single Bond Plus (SB).*

Microleakage

AP showed the highest ($p<0.05$) dye penetration (2.9±0.2 mm) compared with all other materials, while the least dye penetration ($p<0.05$) was exhibited by OB (1.2±0.1 mm). However, there was no statistically significant difference between OB, control group SB (1.4±0.1), and S3 (1.6±0.2). Correlation analysis revealed a strong reverse relation between dye penetration and μTBS values (Pearson coefficient=0.642, $p<0.01$).

Micromorphological analysis of the adhesive-dentin interfaces of the control group SB (Figure 3f) showed a much thicker hybrid layer (over 8 μm) and long resin extensions in the lateral branches of the dentinal tubules when compared to all-in-one products, which in most of the cases exhibited thin hybrid layers not exceeding 1 μm and short resin tags not exceeding 5 μm except for OB (Figure 3a), which showed a resin infiltration similar to that of the control group. Gap formation and separation between the adhesive layer and the composite was identified in most of the samples. Correlation statistics revealed no direct relation between the μTBS and the thickness of the hybrid layer or the length of the resin tags for any of the adhesives.

DISCUSSION

Five all-in-one bonding agents and one etch-and-rinse adhesive were evaluated. The results of the present study led to rejection of all the null hypotheses. Thermocycling and cavity configuration had a significant effect on the μTBS. The DC was correlated with a decrease of μTBS after thermocycling, and there was a strong inverse relation between microleakage and μTBS in class II gingival floor dentin.

In all three different testing conditions, etch-and-rinse control (SB) showed better bonding efficiency than most of the all-in-one adhesives tested. The results of the present study fit well with previous data showing that one-bottle self-etch adhesives are inferior in bonding efficiency to etch-and-rinse products.[15] Several explanations—such as weakened adhesion of the restorative resin composite to the adhesive layer due to a high-oxygen inhibition layer and high acidity,[16] incomplete wetting and insufficient thickness of the adhesive layer, difficulty evaporating residual solvents, and phase separation between hydrophilic and hydrophobic ingredients and the resulting sensitivity to hydrolysis—have been seen as contributing to lower bonding performance of one-bottle all-in-one self-etch adhesives to dentin as compared to the three-step etch-and-rinse and two-step self-etch adhesives.[7,17-19]

Today's adhesives are complex mixtures of functional and cross-linking monomers, curing initiators, inhibitors or stabilizers, solvents, and often silica fillers. The performance of the all-in-one adhesives tested varied, depending on the testing conditions. Differences in their composition and application mode seem to be the key reasons for the different performance of the adhesives tested.[3]

Many studies investigating the performance of self-etch adhesives appear in the literature. However, most of the studies used no thermal or mechanical stresses and do not take into account polymerization stresses and cavity configuration.[20]

In the current study, μTBS was tested and compared on flat dentin surfaces with (flat TC) and without thermocycling (flat non-TC). Then μTBS was evaluated in class II composite resin restorations gingival floor dentin (class II TC) and compared with the bond strength on the flat dentin surfaces (flat TC). In both cases, samples were subjected to thermocycling under similar conditions. To ensure

minimal variations in polymerization shrinkage stresses, standard class II cavities were made, and the same composite resin was used in all groups (flat surfaces and class II cavities). Orientation of the slices and the size of the sticks were standardized so that the bonded areas tested were identical.

According to the results, thermocycling and cavity configuration affected the bonding efficacy of all adhesives tested, even the control two-step etch-and-rinse adhesive (SB). The μTBS values were further significantly decreased in class II restorations after thermocycling for all adhesives tested. The possibility of improper drying of etched dentin should be considered because of the narrower dimensions of the class II cavities prepared in this study. High polymerization contraction stresses, along with high difficulty in removing residual solvents adequately from these hydrophilic adhesives, may be the main reason for this inferior bonding performance in class II restorations.[10]

All-in-one adhesive S3 showed the highest bonding efficiency of all the all-in-one self-etch products tested. S3 exhibited the least reduction in bond strength after thermocycling. It is interesting to notice that S3 on flat surfaces showed inferior bond strength compared to control group SB, but when evaluated on class II restorations after thermocycling, no significant statistical difference was recorded in μTBS values between S3 and control group SB. The findings of our study are in accordance with previous studies comparing S3 with other all-in-one adhesives that reported good bond strength to enamel and dentin even under cyclic loading and mechanical stress.[7,30,31] S3 adhesive is characterized by mild acidity and containing 10-methacryloxydecyl dihydrogen phosphate (10-MDP) in its composition as a functional monomer. This specific molecular composition is capable of interacting with residual hydroxyapatite within the hybrid layer, forming a stable MDP-Ca salt deposition and a strong nanolayer at the adhesive interface.[33] This chemical interaction acting synergistically with superior infiltration into the decalcified substrate, the mild acidity of S3, the homogeneity of the adhesive, and the lack of phase separation might be responsible for enhanced bond stability over time.[15,35]

Low bond strengths were identified for the remaining all-in-one adhesives, especially in class II restorations after thermocycling. An interesting finding was that the modes of failure for all self-etch adhesives tested were mostly mixed on flat surfaces, but in class II gingival floor dentin, an increase in adhesive mode of failure was identified, which might be related to low bond strength.

IB and XB provided the lowest mean bond strength among all adhesives tested regardless of thermocycling. Our study confirmed the poor results of these products seen in previous studies.[39,40] Previous reports show high pretesting failures reaching up to 51.1% for IB, which was related to poor collagen infiltration. IB is an acetone-containing non–2-hydroxyethyl methacrylate adhesive that has been associated with severe phase separation, leading to porosities or blisters occurring at the bonding interface.[15,44,45]

A low DC of dental adhesives has been associated with monomer elution and possible continuous demineralization of dentin, low bond strength values, increased permeability, and phase separation.[48-50] Incomplete polymerization of the adhesive can accelerate the water-degradation effects, leading to bonding deterioration.[51-53]

In most of the studies, degree of conversion evaluation was performed in specimens polymerized on a glass coverslip or an inert surface and not in contact with the bonding structure. In the present study, cured adhesive films fixed on dentin were rinsed with acetone to remove the O_2-inhibition layer from the surface of the adhesive film before FTIR measurements. The DC was evaluated after applying each adhesive on a dentin substrate and not on a glass coverslip or directly onto the instrument's minicrystal, closer to the clinical situation. The quantitative measurement of DC inside the hybrid layer can provide some information to explain current adhesive performance. Self-etch adhesives show a better DC when placed on dentin than on an inert surface.[15]

Differences in DC among the materials tested were observed. A number of reasons might be responsible, such as the resin and filler composition, the initiating system, filler loading, and type of solvent. Acetone-containing adhesives, such as OB and IB tested in the present study, may achieve lower DC compared to the ethanol-containing adhesives.[54,55] High volume of water or solvents makes the viscosity low and leads to a decrease in conversion and an increase in O_2 inhibition.[8]

In the present study, a moderate correlation between DC and bond strength was identified. In the literature, contradictory results have been reported by studies evaluating the effect of DC on bond strength. Two studies evaluating the correlation between μTBS and DC of adhesive systems

failed to find any correlation.[49,53] Another study demonstrated that increased DC is related to an increase in the quality of the polymer network and thus less nanoleakage and higher μTBS.[56] Bond strength is multifactorial, and other factors, such as the composition of the material, are involved in the performance of the adhesives.

Although a negative correlation between dye penetration and μTBS was identified in many studies,[58] a moderate correlation was found between dye penetration and bond strength on the class II gingival floor dentin in the current study. This finding can be attributed to measuring the two parameters using the same sample, which may allow for correlation that is more consistent. AP showed the highest dye penetration in comparison to all other materials and at the same time the lowest bond strength values. The least dye penetration was exhibited by OB, but there was no significant difference between OB, S3, and control group SB. Those three adhesives presented good bonding performance as well.

Micromorphological evaluation of the dentin-adhesive material interface of the samples under SEM with high-magnification imaging showed no direct relation between the depth of infiltration in dentin and the thickness of the hybrid layer or the length of the resin tags for any of the adhesives. An interesting finding was that AP and XB, which exhibited gaps and separation between the adhesive and the resin composite, showed a high number of pretesting failures in class II restorations and a high number of adhesive mode failures. On the contrary, S3 presented a very thin hybrid layer and very few resin tags but no interfacial gaps, and this may explain the results of microleakage resistance and bonding efficiency of S3, which were comparable with the etch-and-rinse control adhesive.

It seems that many factors, such as degree of infiltration of the resin monomers into the collagen, porosity due to blisters at the bonding interface, and hydrolytic degradation of the resin components in the hybrid layer, are related to the integrity of the bonding process.[60]

CONCLUSIONS

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

1. The control etch-and-rinse adhesive showed better bonding efficiency and dye penetration resistance in all testing conditions than the all-in-one self-etch adhesives tested.
2. The μ-TBS of single-bottle adhesives varies significantly due to wide variations in their chemical composition.
3. DC, thermocycling, and cavity configuration had a significant effect on the bond strength of both etch-and-rinse and self-etch products.
4. A good correlation exists between dye penetration and bond strength when the tested adhesive systems are bonded to the class II gingival dentin floor.

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Human Subjects Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Sharjah. The approval code for this study is 111010.

Conflict of Interest

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

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In Vitro Effects of Resin Infiltration on Enamel Erosion Inhibition

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Clinical Relevance

While patient compliance is key to preventive measures related to dental erosion, the application of resin-based materials could serve as an alternative treatment for inhibiting erosion progression.

SUMMARY

Resin-based materials that show promising effects for preventing the progression of erosion have been studied. This *in vitro* study evaluated the effects of applying resin-based materials, including resin infiltration, on previously eroded enamel subjected to erosive challenges. The influence of enamel surface etching prior to application of the material was also studied. Bovine enamel blocks were immersed in hydrochloric acid (HCl), 0.01 M (pH 2.3), for 30 seconds in order to form a softened erosion lesion. The blocks were then randomly divided into nine groups (n=12) and treated as follows: C = control without treatment; Hel = pit & fissure resin sealant (Helioseal Clear); Adh = two-step self-etching adhesive system (AdheSe); Tet = two-step conventional adhesive system (Tetric N-bond); and Inf = infiltrant (Icon). The Helno, Adhno, Tetno, and Infno groups received the same materials without (or with no) surface conditioning. The depth of the material's penetration into softened erosion lesions was qualitatively analyzed using reflection and fluorescence confocal microscopy. After application of the materials, the blocks were immersed in HCl for two minutes; this step was followed by immersion in artificial saliva for 120 minutes four times a day for five days (erosive cycling). Both the enamel alteration and material thickness were analyzed using

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profilometry, and the results were submitted to Kruskal-Wallis and Dunn tests ($p>0.05$). Images from the confocal microscopy showed minimal penetration of Adh/Adhno and deep penetration of Inf/Infno into the erosive lesions. The groups Hel, Adh, Inf, Tetno, and Infno resulted in the formation of a layer of material over the enamel, which was effective in inhibiting the progression of erosion. In conclusion, the infiltrant, with or without etching, was able to penetrate and protect the enamel against dental erosion. The other resin-based materials, except for the two-step conventional adhesive, were able to penetrate and inhibit the progression of erosive lesions only when they were applied after enamel etching.

INTRODUCTION

Dental erosive wear has become a more prevalent and increasing clinical concern.[1-4] The ideal treatment for arresting the development of erosion is to eliminate the cause, which is not always practical or achievable.[5] For this reason, most studies related to the prevention and treatment of dental erosive wear have focused on various fluoride compounds.[6-10] However, there are controversial findings in the literature related to the effectiveness of fluoride in terms of reducing or preventing erosive tooth wear.[11-13] Another proposed therapy is the use of resin-based materials over the dental tissue, which serves as a mechanical barrier between the enamel/dentin and the acidic attack.[14] In a series of *in vitro*, *in situ*, and clinical studies,[15-19] a research group investigated the protective effects of resin-based sealants and adhesives against dentin erosive wear. In summary, coating the dentin with a resin-based bonding agent resulted in a protective effect that lasted for up to three months.[18] On the other hand, the use of a fissure sealant to protect palatal dentin surfaces showed the prevention of tooth wear for up to nine months.[19] Recently, Wegehaupt and others[20] evaluated *in vitro* the long-term protective effects of surface sealants against enamel erosive wear by hydrochloric and citric acids. The resin-based surface sealants tested reduced the enamel loss under long-term (28-day) acid exposition.[18]

Resin infiltration is a new approach that was developed to counteract incipient enamel caries lesions.[19-27] In contrast to conventional sealants, in which the material adheres to the enamel surface, resin infiltration penetrates into the porous lesion body of enamel's initial carious lesions using a special low-viscosity resin that blocks the diffusion of acids into the lesion, thereby slowing or arresting the progression of caries.[21-29] Reflecting back, resin-based materials, such as pit & fissure sealants and adhesives, were not developed to seal erosive lesions, and since resin infiltration blocks the demineralizing effects of cariogenic acids, it is important to note the effects of resin infiltration on erosive lesions. However, the manufacturer contraindicates its use for erosion, regardless of whether anything has been reported in the literature related to the use of resin infiltration to treat erosive lesions. To promote resin infiltration in the resin infiltration system, hydrochloric acid (HCl) is used to remove the hypermineralized superficial layer of the carious lesion.[30] Nevertheless, there remains a concern about the possibility of removing the softened eroded enamel, which could impair the resin infiltrant adhesion, compromising its possible effects against erosion.

Therefore, the present study evaluated the effects of the application of resin-based materials, including resin infiltration, on previously eroded enamel subject to erosive challenges. The influence of eroded enamel surface conditioning prior to material application was also studied. The hypothesis of this study was that all of the resin-based materials that were evaluated would be able to protect eroded enamel against erosion and that enamel etching would not interfere with this effect.

METHODS AND MATERIALS

Experimental Design

This blinded study evaluated the preventive effects of four resin-based materials (pit & fissure resin sealant, Helioseal Clear; self-etching adhesive, AdheSE; conventional adhesive, Tetric N-bond; and the infiltrant Icon) against the progression of dental erosion. Each material was applied with and without enamel superficial conditioning and compared to the control (enamel without resin-based material application). The enamel samples were initially eroded and randomly divided into the studied groups (each group, n=12) for resin-based materials application. The erosive challenge was conducted for five days. The response variable was profilometry (blind analysis for the studied materials). Two additional specimens per group were used to illustrate penetration of the resin-based materials into the eroded enamel using confocal microscopic visualization.

Sample Preparation

Freshly extracted bovine teeth were sectioned at the cementum-enamel junction with a water-cooled,

Table 1: *Resin-based Materials Group, Composition, and Application Steps According to the Manufacturer's Instructions*

Material	Group	Composition	Application Steps
Helioseal Clear (Hel)	Pit & fissure resin sealant	Bis-GMA, TEGDMA, and additives	37% phosphoric acid (30 s), rinsing, and drying; helioseal clear (15 s) and polymerization
AdheSE (Adh)	Self-etching adhesive system	AdheSE Primer: acrylate acid phosphoric, bis-acrylamide derivate, and additives; AdheSE Bond: dimethacrylate, HEMA, silicic dioxide, initiators, and stabilizers	AdheSe-primer (30 s); drying; AdheSe-bond (5 s); drying and polymerization
Tetric N-bond (Tet)	Conventional adhesive system	Bis-GMA, ethanol, HEMA, acrylate phosphonic acid, glycerin dimethacrylate, urethane dimethacrylate, di-tri-phenyl-phosphine-methyl benzoyl, and additives	37% phosphoric acid (30 s), rinsing and drying; tetric N-bond (5 s); drying and polymerization
Icon (Inf)	Infiltrant	Icon-etch: hydrochloric acid, pyrogenic silicic acid, surface-active substances; Icon-dry: 99% ethanol; Icon-infiltrant: methacrylate-based resin matrix, initiators, and additives	Etching with 15% hydrochloric acid (120 s), rinsing and drying; 95% ethanol- and air-drying; resin infiltration with a syringe (180 s); polymerization; and infiltrant reapplication (60 s) plus polymerization

Abbreviations: Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate.

diamond-coated disc (Extec Corp, Enfield, CT, USA) using an ISOMET low-speed saw cutting machine (Buehler Ltd, Lake Bluff, IL, USA). The crowns were individually placed, enamel surface down, in a silicone cylindrical mold with an inner diameter of 5.6 cm, and these crowns were embedded in acrylic resin (Jet Ltd, Campo Limpo Paulista, SP, Brazil). After removing the samples from the mold, they were ground flat with water-cooled silicon carbide discs (320, 600, and 1200 grades of Al_2O_3 paper; Buehler Ltd) and polished with felt paper wet by diamond spray (1 μm; Buehler Ltd). The loss of enamel during the grinding steps was controlled with a micrometer (Mitutoyo, Tokyo, Japan) to be approximately 200 μm. The samples were cleaned using an ultrasonic device for 10 minutes and were then checked microscopically (40×; Carl Zeiss Micro-imaging GmbH 37081, Göttingen, Germany) for the presence of white spots and cracks.

A surface Knoop hardness (KHN) test was performed (five indentations in the center of the slab spaced 200 μm apart, 25*g*, five seconds; HMV-2000; Shimadzu Corporation, Tokyo, Japan) to select 200 bovine enamel blocks (SHi) with hardness values between 317 and 388 KHN (mean surface hardness of 353 ± 17 KHN). The bovine enamel samples were then subjected to short-term acidic exposure by immersion in 0.01M HCl (pH 2.3) for 30 seconds (17.6 mL per block), resulting in surface softening without tissue loss.[31] The surface hardness determination was performed again (SHd) with five measurements localized at a distance of 100 μm in relation to the initial indentations for the final selection of 108 enamel samples with initial erosive lesions (hardness values between 149 and 193 KHN [mean surface hardness of 171 ± 11 KHN]).

After selection, the materials were applied according to the manufacturers' instructions (Table 1). On groups without superficial enamel etching for both the pit & fissure resin sealant and the conventional adhesive system, the enamel was not etched with 37% phosphoric acid gel. On samples from the self-etching adhesive system group, the enamel was not conditioned with AdheSE Primer. Finally, on the infiltrant group, the enamel was not conditioned with 15% HCl gel.

Erosive Cycling

The samples were subjected to five days of erosive cycling by immersion in 0.01M HCl, pH 2.3 (17.6 mL per sample), for two minutes at 37°C under constant motion, followed by immersion in artificial saliva (17.6 mL per sample) for two hours. This cycle was repeated four times per day, and at the end of each day, the samples were stored overnight (14 hours) in artificial saliva.[32] The composition of the artificial saliva that was used was 0.33 g KH_2PO_4; 0.34 g Na_2HPO_4; 1.27 g KCl; 0.16 g NaSCN; 0.58 g NaCl; 0.17 g $CaCl_2$; 0.16 g NH_4Cl; 0.2 g urea; 0.03 g glucose; 0.002 g ascorbic acid; 2.7 g mucin in 1000 mL of distilled water; and pH 7.0.[33]

Profilometric Analysis

Prior to treatment, identification marks were made on the sample surfaces using a scalpel, which allowed for accurate repositioning of the stylus. Subsequently, five baseline surface profiles were

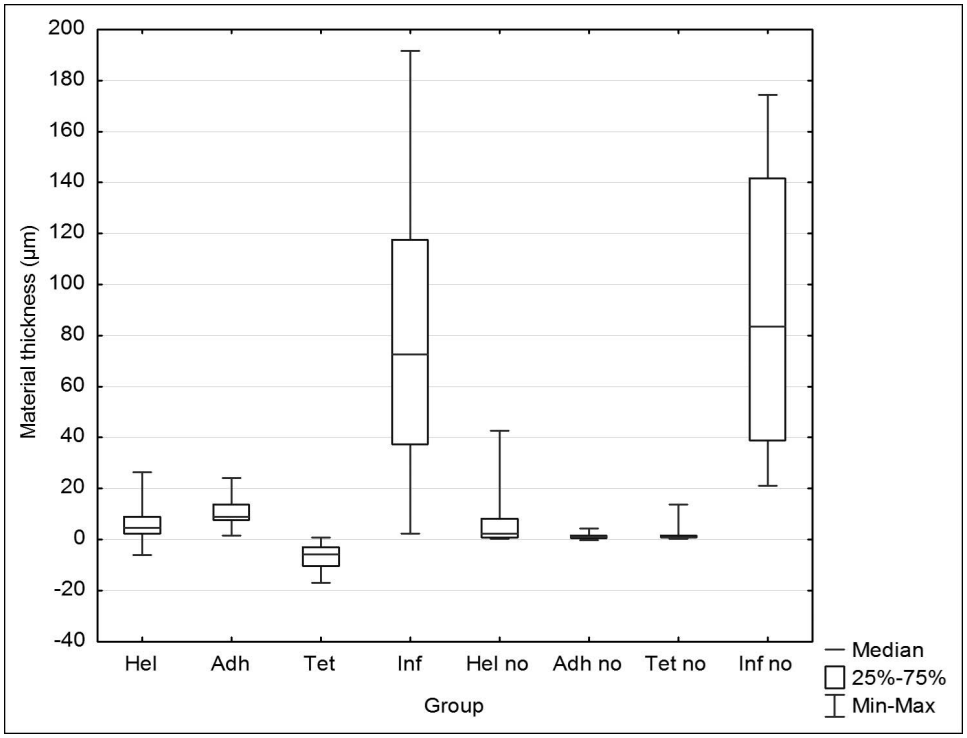

Figure 1. *Median, interquartile range, minimum and maximum values of the resin-based material thickness after application (µm). Nomenclature (Hel = pit & fissure resin sealant, Helioseal Clear; Adh = two-step self-etching adhesive system, AdheSE; Tet = two-step conventional adhesive system, Tetric N-bond; and Icon = infiltrant, Icon). Only the name of the material = application according to manufacturer's instruction. The name of the material + no = application without enamel etching. Median followed by distinct lowercase letters represents the significant difference among the groups, considering material thickness (Kruskal-Wallis and Dunn test, p<0.05).*

obtained from all of the samples as references using a profilometer (MarSurf GD 25, Göttingen, Germany) at a certain distance: 2.25, 2.0, 1.75, 1.5, and 1.25 µm. The marks and two-thirds of the enamel surface were covered with nail varnish and the resin-based materials were applied. The nail varnish was removed, and profilometric analysis was performed again at the same sites used for the baseline measurements. Then, after recovering the marks with nail varnish, the samples were subjected to erosive cycling. The nail varnish was subsequently removed to enable another profilometric analysis. The resin-based material thickness after application and material and/or enamel loss after erosive cycling were quantitatively determined using specific software (MarSurf XCR 20) by calculating the average thickness of the materials and the depth of the eroded surface relative to the baseline surface profiles, respectively. Since the enamel samples could be precisely repositioned in the wells of the profilometer, it was possible to match the respective baseline and final profiles.[34]

Confocal Microscopy Analysis

A 0.05 mg/mL ethanolic solution of tetramethylrhodamine isothiocyanate (Sigma-Aldrich, Steinheim, Germany), was used to label the materials under study by adding 0.02 mL of this solution in 0.5 mL of the material.[35,36] The treatments were performed according to each group (two eroded enamel specimens per group), following the manufacturers' instructions. Resin penetration was observed using confocal laser scanning microscopy (CLSM; LEICA TCS SPE, Leica Microsystems CMS, Mannheim, Germany); the microscope was equipped with four solid-state lasers from 488 to 635 nm. The specimens were observed using a 40× objective in fluorescence (wavelength λ=532 nm) and reflection (wavelength λ=488 nm) modes.

Statistical Analysis

Statistical analysis was performed using SigmaPlot version 12.3 (2011 Systat Software GmbH, Erkrath, Germany). The assumptions of normal distribution of errors (Shapiro-Wilk test) and equality of variances were checked. Since the assumptions were not satisfied, the Kruskal-Wallis test and the Dunn post hoc test were applied. The significance level was set at 5%.

RESULTS

The thickness of the studied materials over the enamel specimens is provided in Figure 1. The thickest layer resulted from the application of resin infiltrant; however, there was no significant difference compared to the self-etching adhesive and pit & fissure sealant

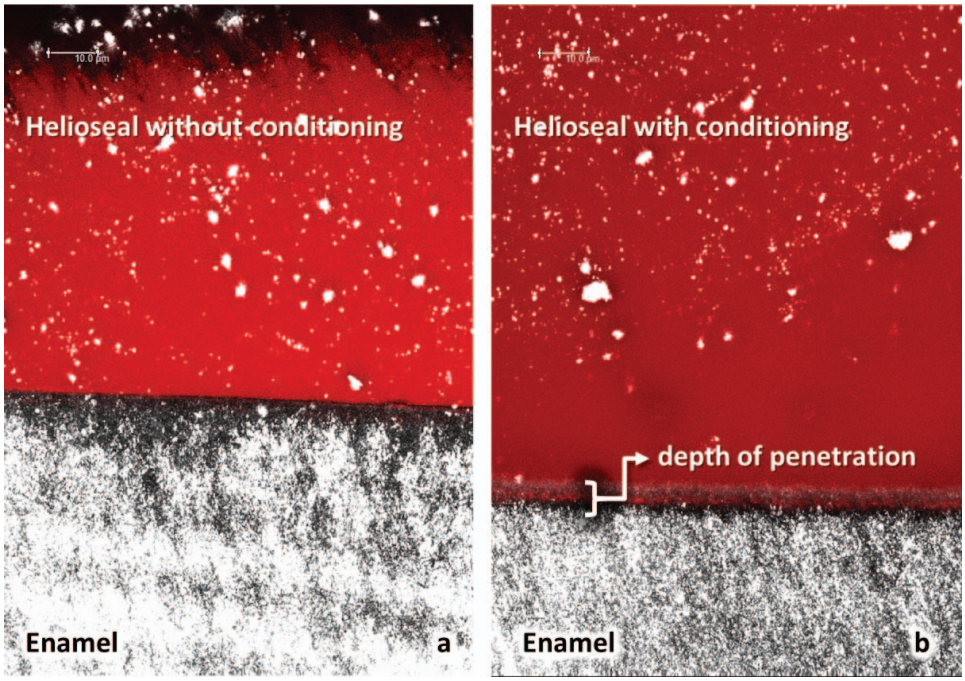

Figure 2. *CLSM pictures of penetration of pit & fissure sealant, Hel. (a) Without enamel etching; (b) with enamel etching.*

when enamel etching was performed. After application of the conventional adhesive with previous enamel etching, negative values, which represent the absence of material over enamel and even enamel loss, were observed. This group showed no significant differences between the same material and the self-etching adhesive without conditioning, since both materials showed a very thin layer of material.

CLSM pictures showed the presence of pit & fissure sealant over enamel regardless of the enamel etching (Figure 2a,b); however, material penetration was observed only when the enamel was etched (Figure 2b). In Figures 3a and 4a it was possible to observe the absence of enamel etching in the self-etching adhesive and conventional adhesive groups, which resulted in the absence of penetra-

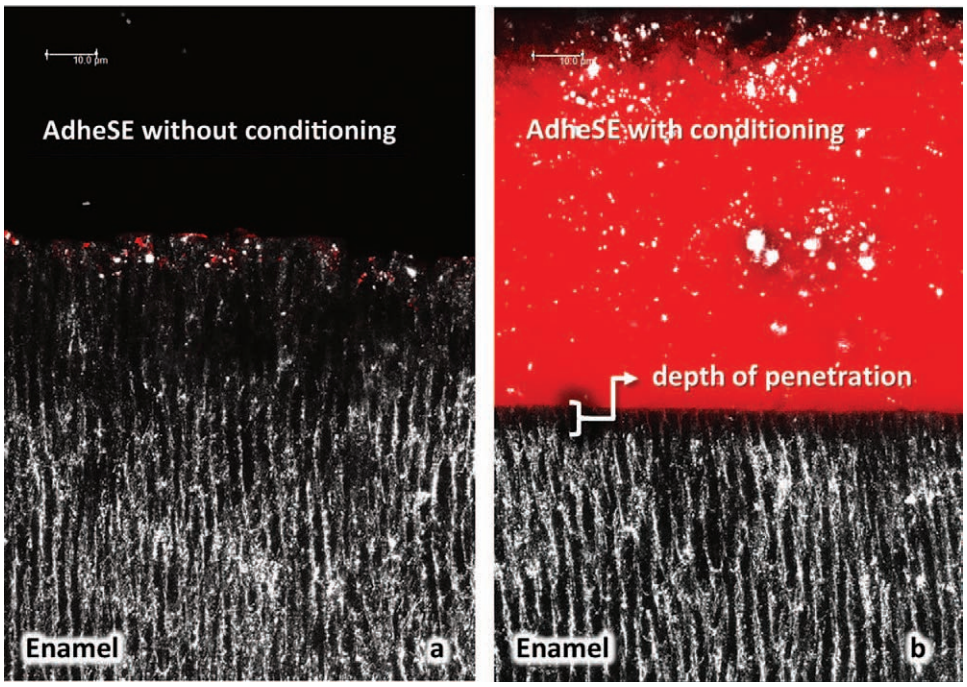

Figure 3. *CLSM pictures of penetration of two-step self-etching adhesive system, Adh. (a) Without enamel etching; (b) with enamel etching.*

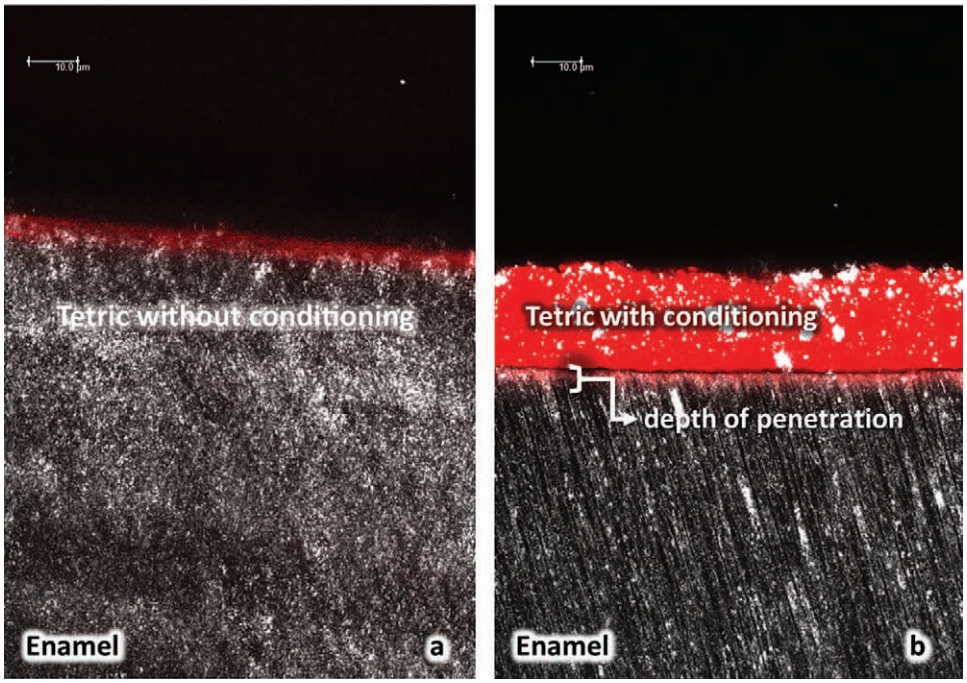

Figure 4. *CLSM pictures of penetration of two-step conventional adhesive system, Tet. (a) Without enamel etching; (b) with enamel etching.*

tion and material. On the other hand, groups in which primer or phosphoric acid was applied showed a thin layer of material penetration into the enamel (Figures 3b and 4b). The resin infiltrant showed the deepest material penetration (Figure 5a,b), especially when hydrochloric acid was used (Figure 5b).

All of the resin-based materials applied with previous conditioning provided enamel protection against erosive cycling, except for the conventional adhesive (Figure 6). On specimens in which the enamel was not conditioned, enamel loss similar to that of the control group ($p>0.05$) could be seen; only on the resin infiltrant and conventional adhesive

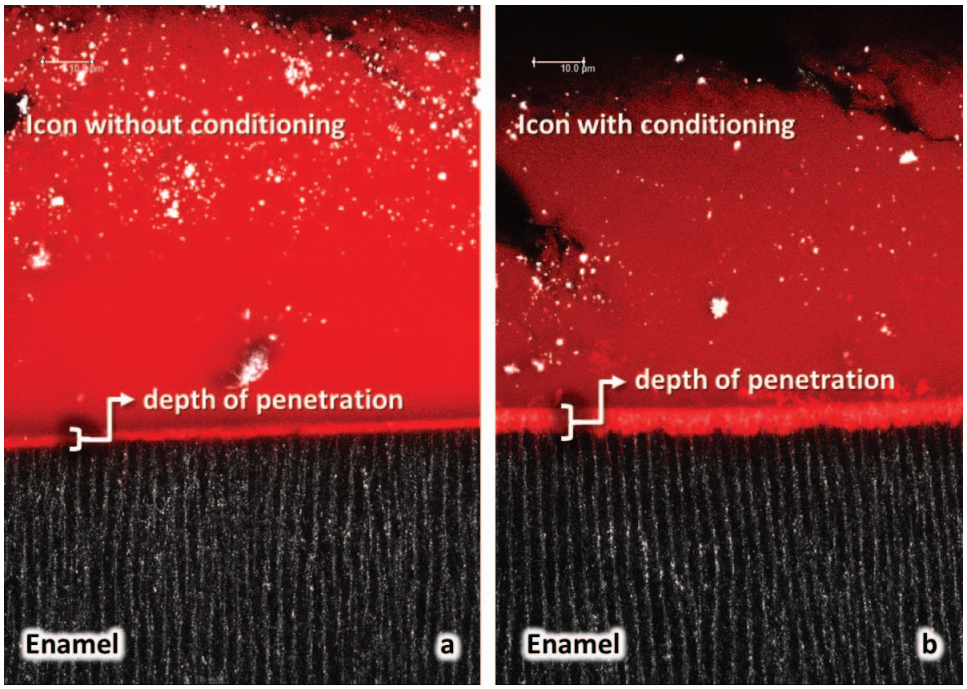

Figure 5. *CLSM pictures of penetration of infiltrant, Icon. (a) Without enamel etching; (b) with enamel etching.*

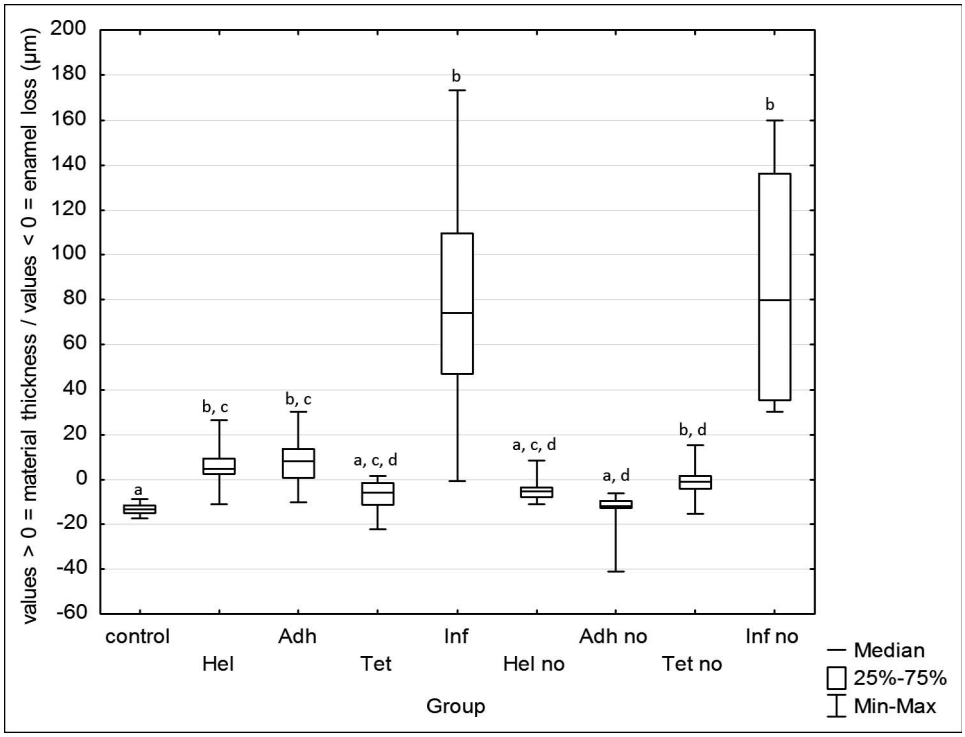

Figure 6. *Median, interquartile range, minimum and maximum values of the material and/or enamel loss after erosive cycling (µm). Nomenclature (Hel = pit & fissure resin sealant, Helioseal Clear; Adh = two-step self-etching adhesive system, AdheSE; Tet = two-step conventional adhesive system, Tetric N-bond; and Icon = infiltrant, Icon). Only the name of the material = application according to manufacturer's instruction. The name of the material + no = application without enamel etching. Median followed by distinct lowercase letters represents the significant difference among the groups, considering enamel loss (Kruskal-Wallis and Dunn test, p<0.05).*

groups was the material maintained on the enamel, preventing enamel wear (Figure 6).

DISCUSSION

The application of resin-based materials preceded by enamel etching, except for the conventional adhesive, protected the enamel against the progression of erosion. However, it is important to note that this result was obtained from an *in vitro* protocol with a five-day erosive challenge. According to Wegehaupt and others,[20] an erosive time of six minutes with HCl (pH 3.0) simulates one day of an intraoral clinical situation, since, in gastroesophageal reflux patients, the pH drops below 5.5 for 4.3 minutes during 24 hours.[37] In the present study, the HCl pH was 2.3 and the blocks were immersed for 40 minutes; thus, the cycling protocol might simulate 10 or more *in vivo* days. However, it is not known how these materials might act clinically on erosive challenges of a longer duration, especially with regard to the infiltrant. There are clinical studies that demonstrate the need for reapplication of the adhesive and pit & fissure sealant after three and nine months, respectively, to maintain their preventive effects in relation to dentin wear. Those clinical studies were conducted with other commercial brands, and the materials were applied over dentin, not enamel.[18,19]

The adhesives were not designed to be exposed to the oral environment, since they were developed to enhance the adhesion of resin composites. When used over dental substrate to form a mechanical barrier against the action of acids, similar to resin pit & fissure sealants, their effectiveness is related to their retention and durability. Those characteristics depend on two factors: penetrability into the acid-etched enamel and wear resistance in the oral environment.[38]

Resin infiltration was developed for the conservative treatment of initial carious lesions,[29] which are characterized by a subsuperficial structure of demineralization.[39,40] To promote resin penetration, HCl is used to remove the hypermineralized superficial layer of the carious lesion. On the other hand, the initial erosive lesion corresponds to a superficial softened area[39-41] that might be penetrated by resin infiltration. However, an additional effect of HCl as the infiltrant conditioner on an eroded and softened area could be enamel wear, lower mineral content, or a mechanically less-stable surface. Considering these aspects, resin infiltrant without acid etching was tested. For standardizing purposes, the other materials were also tested without enamel conditioning even though phosphoric acid promotes less enamel alteration when compared to HCl. In the groups where the enamel was etched, and mainly in the infiltrant group, it is likely that the softened layer of the initial erosion lesion was removed. Other studies have reported that enamel etching with

phosphoric acid and HCl resulted in enamel loss of approximately 10 and 15 μm, respectively,[23,38] which corresponds to a thicker layer compared with initial erosion.[39] Even with the possible removal of the erosion lesion, the images obtained with confocal microscopy showed that all materials penetrated into the previously etched enamel, with an emphasis on the infiltrant, which presented a thick, homogeneous, and deep penetration compared to the other studied materials. This greater penetration showed that the remaining enamel presented appropriate characteristics for adhesion (Figure 5). According to Lussi and others,[41] persistent acidic attacks result in substance loss in which the outermost superficial enamel is eliminated and the remaining tissue is softened. This softened tissue reaches equilibrium and there is no further progress, not even with prolonged acidic impact when bulk mineral undergoes further dissolution.[42] Thus, this equilibrated, softened tissue might also show constant characteristics related to enamel adhesion.

Penetration of the infiltrant was an unexpected result that was obtained, even when there was no enamel etching. A possible explanation for this is the type of acid used to develop the initial lesion of erosion—HCl—which corresponds to the indicated acid for enamel etching for the infiltrant. Note that the length of time for which the acid was applied, which can influence the surface characteristics of enamel, was lower in this study (30 seconds × 120 seconds), since the objective was to form an initial erosion lesion (softened surface) without wear. A pilot study was conducted to determine the amount of time required for enamel to soften without enamel loss. This characteristic was assessed by the loss of surface hardness. The parameter used was the loss of sharpness of the indentations' limits after acidic attack at each 15-second interval compared to the indentations performed on sound enamel. In the etching technique for the infiltration of caries, HCl is used for two minutes.

In terms of wear in the oral environment, resin-based materials are subject to two major challenges: acids and mechanical forces. Laboratory studies indirectly and directly found an erosive resistance to adhesives and fissure sealants.[43,44] Even under prolonged *in vitro* erosive challenge, resin-based materials remained on the dental substrate, protecting the dentin against erosion.[43] Different types and brands of adhesives might show different behaviors in terms of adhesion and their ability to protect against enamel erosion. The results of this study show enamel wear when conventional adhesive was applied (Figure 4). It is hypothesized that there were enamel sites that were acid etched but not covered with adhesive. The application of two adhesive layers could compensate for this failure; however, in this instance, one cover layer was applied for standardization purposes. In addition, despite enamel loss after application of the conventional adhesive, substantial additional enamel loss was not observed when the specimens were subjected to erosive challenge, suggesting a protective effect. When comparing thickness of the material after application (Figure 1) with thickness of the same material subjected to erosive challenge (Figure 6), minimal wear was noted for the groups with enamel conditioning. For the pit & fissure resin sealant and self-etching adhesive system without enamel conditioning, the material thickness was significantly less after the acid attack. Nevertheless, this phenomenon was due to the entire material loss, not material wear.

There are no data related to infiltrant resistance to acids. In the present study, the infiltrant was able to protect the enamel. And even after the erosive challenge, the thickness of the infiltrant that covered the enamel surface was nearly the same, regardless of enamel conditioning. In Figure 1, the thick layer of the infiltrant can be seen, which resulted from the mode of application and apparatus provided by the manufacturer. A layer of infiltrant of lesser thickness could have been produced if a microbrush was used, as was the case in the other groups. On the other hand, after 20,000 abrasion cycles, when the infiltrant vs the adhesive applied over the caries lesion was compared with the original enamel (42.6 ± 20.7 μm vs 40.4 ± 18.5 μm, $p > 0.05$), nonsignificant differences in vertical wear loss were measured.[45] However, the infiltrant material showed surface and morphological aspects that pointed to improved surface stability and infiltration quality.[45] Thus, a thicker layer of infiltrant might be beneficial for wear resistance to toothbrush abrasion and might be tested in future studies. Furthermore, color stability of the infiltrant merits further study to assess its potential as a preventive layer for smooth enamel surfaces in esthetic areas.[46]

The resistance of resin-based materials to abrasion from tooth brushing is poorly reported in the literature.[47] Adhesives are mainly studied as composite surface coatings.[48,49] When considering erosion associated with brush abrasion, the results are controversial.[50,51] An *in vitro* study[51] showed that after one year of tooth brushing, significant surface deterioration with deleterious loss of enamel and

discoloration was observed in all four of the tested sealants. The authors emphasized the need for revision of the application of sealants on smooth enamel surfaces.[51] In addition to the promising results of the present study, more studies are necessary to clarify retention and durability of the studied resin-based materials, especially in terms of prolonged erosive and abrasive challenges. Furthermore, before resin infiltration can be tested in clinical situations, it is also important to know the effects of the material on dentin erosion inhibition, since erosive lesions are frequently diagnosed at an advanced stage when the dentin is compromised.

CONCLUSIONS

Infiltrant applied with or without enamel etching was able to penetrate and protect enamel from dental erosion. Other resin-based materials, except for two-step conventional adhesives, were able to penetrate and inhibit the progression of erosive lesions only when applied after enamel etching. However, especially in the case of infiltrant, this was the first step to evaluating the infiltrant's ability to prevent the progression of erosion, and further studies will be necessary before these results can be extrapolated for clinical use.

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Human Subjects Statement

This study was conducted at the Bauru School of Dentistry, University of São Paulo.

Conflict of Interest

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

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Evaluation of Cytotoxicity of Dentin Desensitizing Products

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Clinical Relevance

Colgate Sensitive Pro-Relief, Topex and Clinpro White Varnish could be recommended in the treatment of dentin hypersensitivity with regard to their cytocompatibility.

SUMMARY

Objectives: To evaluate the cytotoxic effects of the dentin desensitizing products (DDPs) used in the treatment of dentin hypersensitivity on cultured human gingival and pulpal fibroblast cells.

Methods and Materials: The cytotoxic effects of DDPs (Smart Protect, Systemp Desensitizer, Seal & Protect, Aqua-Prep F, Isodan, Gluma, BisBlock, D/Sense Crystal, UltraEZ, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish) on cultured human gingival- and pulp-derived fibroblast cells were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test (Serva, Heidelberg, Germany) under two different conditions. In the first test, different dilutions of the DDPs were directly applied onto cultured gingival fibroblast cells, and in the second test, the products were applied onto different-thickness dentin discs (0.5 and 1 mm) placed above cell culture medium, which contained pulp fibroblast cells.

Results: According to the cytotoxicity evaluations of gingival fibroblast cells, the cytotoxicity of all of the DDPs was very high at 50% concentrations ($p<0.05$). Colgate Sensitive Pro-Relief, Clinpro White Varnish, and Topex showed higher cytotoxicity than did the other products ($p<0.05$), decreasing with further dilutions, and these products were found to be less cytotoxic to both types of cells ($p<0.05$) than were the other products with further dilutions. The cytotoxicity to human gingival and pulpal fibroblast cells of Systemp Desensitizer, Aqua-Prep F, Isodan, and Gluma did not show any decrease with further dilutions, and these products were found to be more cytotoxic than the other products ($p<0.05$).

Conclusions: According to the findings of this study, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were less cytotoxic than the other DDPs used in this study.

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INTRODUCTION

Dentin hypersensitivity (DH) is a frequent dental complaint in the adult population, with a prevalence ranging from 4% to 74%, and it occurs in patients between 18 and 70 years of age.[1,2] DH is defined by

short and sharp pain arising from exposed dentin in response to chemical or physical stimuli, typically evaporative, tactile, or osmotic stimuli, that cannot be explained by any other dental problem.[3] Generally, DH occurs as a result of more than one factor. Gingival recession, enamel, and cementum loss are the prevailing causes of oral exposure of dentinal tubules. Dentinal tubule exposure is mainly seen in the cervical regions of the vestibular faces of the teeth.[4,5] Open dentinal tubules provide a direct link between oral environmental factors and the pulpal tissues.[6] Although several theories have been proposed to explain the mechanism of DH, the most widely acknowledged theory is the hydrodynamic theory, proposed by Branström and co-workers. According to this theory, a chemical or physical stimulus allows for dentinal fluid movement within the dentinal tubules in the presence of open dentinal tubules. The theory further proposes that the fluid movement affects the pulpal mechanoreceptors, causing the sensation of pain.[7-9]

Clinical management of DH is based on the prevention or elimination of possible causes of pain. Dentinal tubule occlusion and blockage of nerve activity using desensitizing products are common methods of treatment of DH.[10] Thus, many dentin desensitizing products (DDPs) with different ingredients have been introduced to the market in different forms.

Professional desensitizing products include chemical agents, such as fluoride,[11] oxalates,[12] calcium compounds,[13] potassium nitrate,[14] strontium salts,[15] glutaraldehyde,[16] adhesive materials,[17,18] and arginine-containing desensitizers.[19-22] The desensitizing effects of many of these materials can be reduced over time by refraining from an acidic diet and through daily tooth brushing. Therefore, the success of long-term treatment of DH is thought to depend on occluding and penetrating the dentinal tubules to resist acid attacks as well as on tooth brushing.[23]

In addition to their desensitizing effects, it has been shown that many of these DDPs contain several cytotoxic components, such as glutaraldehyde, 2-hydroxyethyl methacrylate (HEMA), triclosan, resin monomers, and sodium fluoride.[24-27] Some of the desensitizing products on the market that contain resin monomers have similar content to dentin bonding agents. After being applied on the gingiva, bonding agents caused pathological changes in the oral mucosa.[28] When bonding agents come into direct contact with fibroblast cells, negative effects have been found in different *in vitro* studies.[26] For instance, HEMA, which is found in many DDPs, was reported to cause abnormal morphological development and inhibition of cell reproduction in cultured human epithelial cells and pulpal fibroblasts.[24] The cytotoxicity of fluoride, which is frequently used in toothpastes and gargles, in DH treatment has also been reported.[25,29] Fluoride has a repressive effect, particularly on protein synthesis and the mitochondrial activities of human pulpal cells.[30] Glutaraldehyde, which is added to desensitizers to reduce dentin permeability, also has the effect of coagulating protein structures.[16]

Similarly, in clinical practice, application of these products to the cervical area of a hypersensitive tooth can result in contact with the gingival tissues. In addition, these products pose significant potential toxicity to the pulpal tissues by moving from open dentinal tubules into the pulp.[26] Hence, evaluating the possible cytotoxic effects of these products on gingival and pulpal tissues is important for the health of the teeth and gingival tissues. Although there have been various studies on the effects, mechanisms, and clinical effectiveness of DDPs, the number of studies concerning their biocompatibility has been very limited.[31,32]

The biocompatibility of dental materials has been evaluated using primary human cells, such as periodontal ligament and pulp fibroblast cells, gingival fibroblasts, and odontoblast-like cells, and permanent nonhuman cell lines, such as 3T3 and L-929, as well as primary and immortalized bovine dental papilla-derived cell lines.[23,27,32-36]

In this study, we aimed to evaluate the cytotoxicity of DDPs commonly used in clinical settings for the treatment of DH, and we used an assay system that enabled more clinically relevant assessment of toxicity, in that dentin barriers with different thicknesses were placed between the DDPs and the pulpal fibroblasts, and the cytotoxic effects of the DDPs on the pulpal fibroblasts were assessed by setting a simple mechanism in tissue culture wells.

METHODS AND MATERIALS

Materials

Twelve different DDPs were used in this study, and their brand names, compositions, and batch numbers are shown in Table 1.

Cell Culture

The cytotoxic effects of DDPs on human gingival- and pulp-derived fibroblasts were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-

Table 1: *Ingredients, Manufacturers' Information, and Lot Numbers for 12 Different Dentin Desensitizing Products (DDPs)*

Groups	Agents	Contents	Manufacturers' Information, Lot Numbers
1	Smart Protect	Antibacterial content in aqueous alcoholic solution (2%-10% glutaraldehyde, 20%-30% isopropyl alcohol, triclosan), and 0.14% fluoride	Detax, Ettlingen, Germany, 090201
2	Systemp Desensitizer	5% glutaraldehyde, <0.1% maleic acid, polyethylene glycol dimethacrylate, and water	Ivoclar Vivadent AG, Schaan, Lichtenstein, M74246
3	Seal & Protect	Di- and trimethacrylate resins, pentaacryloyldipentaerythrytol phosphoric acid, photoinitiators, butylated hydroxytoluene, cetylamin hydrofluoride, triclosan, acetone, and functionalized amorphous silica	Dentsply, Detrey, Konstanz, Germany, 0906004007
4	Aqua-Prep F	10%-30% HEMA and 1%-2% NaF	Bisco Inc, Schaumburg, IL, USA, 1000004372
5	Isodan	0%-40% HEMA, 0%-0.5% NaF, 0%-5% potassium nitrate, sherry flavor, and glycerol	Septodont, St Maur des Fosses, Cedex, France, 46061
6	Gluma	35% HEMA, 5% glutaraldehyde, and water	Heraeus Kulzer GmbH & Co, Hanau, Germany, 010092
7	BisBlock	<5% oxalic acid	Bisco Inc, Schaumburg, IL, USA, 1000003731
8	UltraEZ	3% potassium nitrate, and 0.25% NaF	Ultradent, South Jordan, UT, USA, 53
9	D/Sense Crystal	2.5% potassium binoxalade, 2.5% nitric acid	Centrix, Shelton, CA, USA, 91749
10	Colgate Sensitive Pro-Relief	L-arginine, calcium carbonate, glycerin, water, bicarbonate, hydrated silica, and sodium saccharin	Colgate-Palmolive Co, New York, NY, USA, 9323201110
11	Topex Topical A.P.F. Gel	2.7% NaF, sodium saccharin, kaolin, and glycerin	Sultan Healthcare, Hackensack, NJ, USA, 0513100519
12	Clinpro White Varnish	5% NaF	3M ESPE, St Paul, MN, USA, M13340

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; NaF, sodium fluoride.

lium bromide (MTT) test (Serva, Heidelberg, Germany) under two different conditions. In the first test condition, the DDPs, at various concentrations, were directly applied onto human gingival fibroblasts, while in the second condition, the products were applied onto dentin barriers placed above cell culture medium, which contained human pulp fibroblasts.

Both types of fibroblasts were derived from appropriate tissue samples obtained from healthy subjects who consented to tissue donation for the study, which was approved by the Scientific Research Evaluation Commission of the Medical Faculty of Karadeniz Technical University (file no. 2010/82). In brief, pulpal and gingival tissues were cut into small sizes and were used as explants in tissue culture flasks, with a growth medium containing Dulbecco modified Eagle's Medium (Lonza, Basel, Switzerland) and 10% fetal bovine serum (FBS) plus a penicillin/streptomycin/fungizone mixture (Lonza). The explants were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Growing cells were harvested by 0.25% trypsin and were subcultured until passages three and four to use in the following experiments.

Preparation of Dentin Discs

One hundred thirty sound human third molars were used for this study. They were stored at 4°C in distilled water containing 0.2% sodium azide with thymol to inhibit microbial growth until they were used. While hydrated, the crowns of the teeth were cut mesiodistally parallel to the long axis of the teeth to prepare dentin discs with thicknesses of 0.5 ± 0.05 mm and 1 ± 0.05 mm by means of a low-speed precision cutting machine (Micra Cut 125, Metkon, Bursa, Turkey). Dentin discs were examined with an optical microscope (Olympus Metallurgical Microscope), and only dentin discs that were nearest to the pulp tissue but that did not contain any pulp tissue were used. The pulpal surfaces of the dentin discs were marked to prevent

the buccal surfaces of the discs from being confused with the pulpal surfaces. Each specimen was then immersed in ethylenediamine tetraacetic acid (14%) for two minutes to remove the smear layer from both surfaces and was then rinsed under tap water for five minutes.[33] The pulpal surfaces of the dentin discs were then marked again and were kept in a saline solution.

Serial dilutions (50%, 20%, 10%, and 1%) of the products (Smart Protect [group 1], Systemp Desensitizer [group 2], Seal & Protect [group 3], Aqua-Prep F [group 4], Isodan [group 5], Gluma [group 6], BisBlock [group 7], and D/Sense Crystal [group 9]) were used directly in culture medium. In the cases of solid or semisolid products, such as UltraEZ (group 8), Colgate Sensitive Pro-Relief (group 10), Topex (group 11), and Clinpro White Varnish (group 12), 0.1 or 1 g of each product was diluted in 0.1 or 1 mL of culture medium, followed by vortexing and incubation at 37°C and 5% CO_2 atmosphere for 24 hours.[37] The resulting elutions were passed through a 0.45-μm filter and were diluted to 50%, 20%, 10%, and 1% in the culture medium.[33] During this procedure, the medium was protected from light to prevent any polymerization.

Cytotoxicity Tests

Direct Contact of DDPs with Human Gingival Fibroblasts—Cells were diluted in growth medium and were seeded into 96-well plates (1×10^4 cells per well) to obtain subconfluent monolayers of cells, following incubation at 37°C and 5% CO_2 atmosphere for 24 hours. The medium was aspirated from all of the wells and was replaced with 100 μL per well of the test solution (50%, 20%, 10%, 1%), prepared as described above. During the test procedures, the products were protected from light to prevent any polymerization. The plates with control wells (ie, no test product) were incubated for another 24 hours. The contents of the plates were decanted, and the wells were washed twice with culture medium without FBS before adding MTT (0.5 mg/mL final concentration) into all of the wells.

The plates were kept in a CO_2 incubator for three hours. Optical density (OD) was determined by dissolving the MTT-formazan product with dimethyl sulfoxide (Merck, Darmstadt, Germany). Only viable cells owning functional mitochondria are able to reduce MTT to insoluble purple formazan crystals.[38] The spectrophotometric absorbance was measured at 570 and 630 nm using an enzyme-linked immunosorbent assay microplate reader (Sunrise, Tecan, Switzerland).

This experiment was conducted twice. In each experiment, each test product and control group was prepared in quadruplicate. The OD values obtained for the same dilution were recorded and averaged as a single measurement. The mortality percentage of the cells (MP) was calculated from the following formula:[39]

$$\mathrm{MP}\% = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100.$$

Cytotoxicity of DDPs to Pulpal Fibroblasts Using Dentin Discs: Preparation of Silicone Molds—A device was designed to place the dentin barriers in 24-well plates in a stable manner so that they would be able to contact the pulpal fibroblast cells and so that the products could be applied on the dentin discs. The diameter of the device was designed in such a manner that it would be equal to the diameter of one well of a 24-well plate. In addition, in the middle of the upper part of this device, a metal stick was designed, the apex of which was 3×3 mm. The marked pulpal surfaces of the dentin discs, with thicknesses of 0.5 mm and 1 mm, were placed immediately below the metal stick in a manner that would allow them to come into contact with the lower part of the device. The surrounding area of the metal stick was thoroughly filled with hydrophilic vinyl silicone (Bisico S1, Bisico Bielefelder Dentalsilicone GmBH, Bielefeld, Germany).

So that the wells could be easily handled and so that they could be placed in 24 wells, a small hole was created on the silicone mold with a hand tool before the silicone hardened. After the silicone hardened, the upper part of the apparatus was discarded, and the dentin discs that were within the silicone molds were obtained (Figures 1 and 2). Dentin discs with the same thicknesses were randomly divided into 13 groups and were placed in silicone molds using the device in a manner that would yield five samples for each group. The contact areas of the dentin discs with the impression material were tightly and hermetically sealed with dental wax. It was determined that there was no leakage between the dentin discs and impression material. One hundred thirty dentin discs in total, which were 0.5 mm and 1 mm in thickness, were inside the silicone molds and were sterilized with ethylene oxide gas. The toxicity of the DDPs to pulpal fibroblasts was evaluated in 24-well plates seeded with the cells. Dentin discs fitting the diameters of the wells of 24-well plates were pushed carefully with pliers until they contacted the medium from the upper part toward the well.

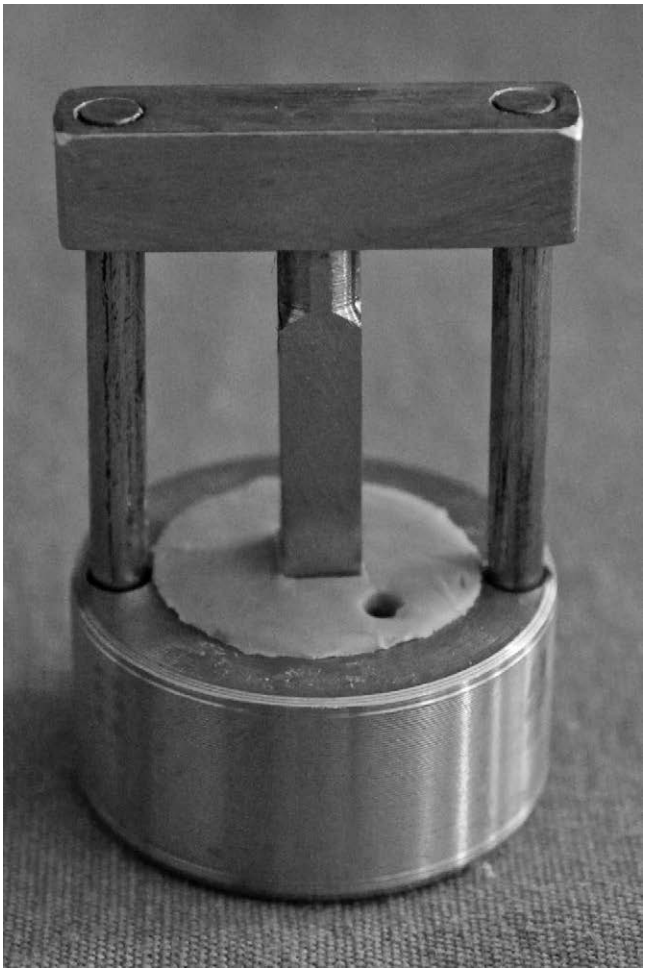

Figure 1. *The device that was designed to hold the dentin discs.*

Each test product was placed in the gap on the dentin barrier without being diluted. The 24-well plates were incubated at 37°C in 5% CO_2 for 24 hours. Dentin discs within the silicone molds were discarded using pliers at the end of the test. The MTT test was performed as described above.

Statistical Analysis of Cytotoxicity

The analysis of the data was performed using statistical software for Windows (SPSS, version 11.5, SPSS Inc, Chicago, IL, USA). The mean and standard deviation (SD) values of MP are shown in Table 2. Multiple comparisons among the groups, in terms of mortality percentage values, were performed by two-way analysis of variance (ANOVA). The significance of the differences between groups within dilutions in gingival fibroblasts was investigated with the Bonferroni correction test. The significance of the differences among groups within

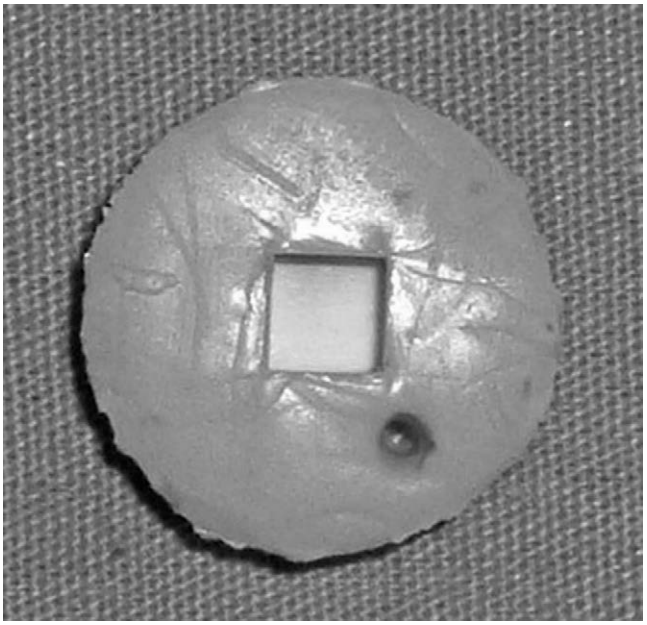

Figure 2. *Dentin discs within silicone molds.*

different-thickness dentin discs in pulpal fibroblasts was investigated with the Fisher least significant difference (LSD) test. Significant differences were found between the material groups and the control groups. The significance level was set at 95% for all of the statistical tests.

RESULTS

Cytotoxicity of Desensitizing Products to Gingival Fibroblasts

Two-way ANOVA revealed significant interactions ($p<0.001$) among the products and concentrations, as shown in Table 2, indicating 1) that cytotoxicity decreased as medium dilution increased and 2) that the toxicities of the products were different.

Table 2: *Two-way Analysis of Variance (ANOVA) Table for Overall Models (Mortality Percentages of Gingival Fibroblasts)*[a]

	Sum of Squares	*df*	Mean Square	*p*
Agents	251,118.06	11	22,828.91	<0.001
Dilutions	58,636.15	3	19,545.38	<0.001
Agents × Dilutions	76,485.76	33	2317.76	<0.001
Error	54,169.01	240	225.705	<0.001
Total	1,798,153.98	288		

[a] *Two-way ANOVA revealed significant interactions (p<.001) among the products and dilutions.*

Table 3: *Cytotoxicity of Products to Gingival Fibroblasts Within Same Dilutions*[a]

Dentin Desensitizing Products	50% Dilutions	20% Dilutions	10% Dilutions	1% Dilutions
1. Smart Protect	92 (2) [A]	85 (1) [AFG]	80 (2) [AC]	49 (2) [AC]
2. Systemp Desensitizer	95 (1) [A]	94 (0) [AFG]	94 (1) [AE]	92 (1) [B]
3. Seal & Protect	93 (5) [A]	77 (3) [G]	73 (3) [BC]	64 (12) [CD]
4. Aqua-Prep F	97 (0) [A]	97 (0) [AF]	97 (1) [DE]	94 (1) [B]
5. Isodan	98 (2) [A]	98 (1) [AF]	97 (1) [DE]	95 (0) [B]
6. Gluma	96 (0) [A]	94 (1) [AFG]	94 (1) [AE]	92 (0) [B]
7. BisBlock	97 (0) [A]	97 (1) [AF]	91 (13) [AE]	55 (29) [AD]
8. UltraEZ	98 (0) [A]	55 (24) [B]	14 (22) [FM]	3 (36) [E]
9. D/Sense Crystal	97 (1) [A]	97 (1) [F]	97 (1) [GE]	63 (21) [AD]
10. Colgate Sensitive Pro-Relief	72 (29) [B]	5 (21) [C]	0 (36) [H]	0 (32) [E]
11. Topex	95 (4) [A]	13 (23) [C]	0 (27) [H]	0 (21) [E]
12. Clinpro White Varnish	72 (22) [B]	18 (8) [C]	7 (18) [HM]	5 (26) [E]

[a] *According to Bonferroni's test, in each column (each tested dilution), values with different superscript letters indicate significant differences ($p<0.05$) among different groups in the same dilutions. The results are expressed as the means of mortality percentages (S.D.). The mean difference is significant at the 0.05 level.*

The MP values (means and SDs) of different groups with different dilutions in gingival fibroblast cells are shown in Table 3.

Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish showed smaller MP values than did the other groups in all dilutions except the 50% dilution ($p<0.05$), and there were no statistically significant differences between each of these products, except in the case of the dilution of 50% ($p>0.05$). The MP values of Systemp Desensitizer, Aqua-Prep-F, Isodan, and Gluma were all greater in each dilution.

Table 4 shows the decreases in the MP values of the products with the dilutions. The MP values of Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish showed statistically significant differences at 20%, 10%, and 1% dilutions, compared to at 50% dilution. The cytotoxicity of Systemp Desensitizer, Aqua-Prep F, Isodan, and Gluma did not show any statistically significant differences among dilutions. Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish was found to be less cytotoxic to gingival fibroblasts in all dilutions, whereas Systemp Desensitizer, Aqua-Prep-F, Isodan, and Gluma were found to be more cytotoxic in all tested dilutions ($p<0.05$).

Cytotoxicity of DDPs to Pulpal Fibroblasts

The MP values (means and SDs) of different groups with different dentin disc thicknesses on pulpal fibroblasts are summarized in Table 5.

Table 4: *Cytotoxicity of Products to Gingival Fibroblasts Within Different Dilutions*[a]

Dentin Desensitizing Products	50% Dilutions	20% Dilutions	10% Dilutions	1% Dilutions
1. Smart Protect	92 (2) [A]	85 (1) [A]	80 (2) [A]	49 (2) [B]
2. Systemp Desensitizer	95 (1)	94 (0)	9 (1)	92 (1)
3. Seal & Protect	93 (5) [A]	77 (3) [AC]	73 (3) [BC]	64 (12) [BC]
4. Aqua-Prep F	97 (0)	97 (0)	97 (1)	94 (1)
5. Isodan	98 (2)	98 (1)	97 (1)	95 (0)
6. Gluma	96 (0)	94 (1)	94 (1)	92 (0)
7. BisBlock	97 (0) [A]	97 (1) [A]	91 (13) [A]	55 (29) [B]
8. UltraEZ	98 (0) [A]	55 (24) [B]	14 (22) [C]	3 (36) [C]
9. D/Sense Crystal	97 (1) [A]	97 (1) [A]	97 (1) [A]	63 (21) [B]
10. Colgate Sensitive Pro-Relief	72 (29) [A]	5 (21) [B]	0 (36) [B]	0 (32) [B]
11. Topex	95 (4) [A]	13 (23) [B]	0 (27) [B]	0 (21) [B]
12. Clinpro White Varnish	72 (22) [A]	18 (8) [B]	7 (18) [B]	5 (26) [B]

[A] *According to Bonferroni's test, in each row (each tested product), values with different superscript letters indicate significant differences ($p<0.05$) among different dilutions in the same groups. The groups that do not contain any letters indicate no significant differences ($p>0.05$) between different dilutions. The results are expressed as the means of mortality percentages (S.D.). The mean difference is significant at the 0.05 level.*

Table 5: *Cytotoxicity of Products to Pulpal Fibroblasts*[a]

Products	0.5 mm	1 mm
1. Smart Protect	98 (2) ^{A,1}	97 (1) ^{A,1}
2. Systemp Desensitizer	98 (2) ^{A,1}	97 (0) ^{A,1}
3. Seal & Protect	58 (11) ^{BD,1}	50 (11) ^{B,1}
4. Aqua-Prep F	94 (1) ^{A,1}	92 (6) ^{A,1}
5. Isodan	98 (0) ^{A,1}	98 (0) ^{A,1}
6. Gluma	97 (13) ^{A,1}	82 (0) ^{A,1}
7. BisBlock	49 (1) ^{BC,1}	30 (1) ^{D,1}
8. UltraEZ	39 (5) ^{C,1}	30 (13) ^{D,1}
9. D/Sense Crystal	70 (21) ^{D,1}	68 (7) ^{C,1}
10. Colgate Sensitive Pro-Relief	26 (21) ^{E,1}	15 (12) ^{E,1}
11. Topex	29 (13) ^{E,1}	12 (13) ^{E,2}
12. Clinpro White Varnish	30 (10) ^{E,1}	11 (8) ^{E,2}

[a] *According to Fisher's least significant difference (LSD) test, in each column (each tested dentin thickness), values with different superscript letters indicate significant differences ($p<0.05$) among different groups with the same thickness. The results are expressed as the means of mortality percentages (S.D.). In each row (each tested agent), values with different numbers indicate significant differences ($p<0.05$) among different thicknesses in the same tested products. The results are expressed as the means of mortality percentages (S.D.). The mean difference is significant at the 0.05 level.*

According to two-way ANOVA and the LSD tests, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were found to be less cytotoxic than the other groups at both thicknesses ($p<0.05$), and no statistically significant differences were detected among these three groups ($p>0.05$). In contrast, Smart Protect, Systemp Desensitizer, Aqua-Prep-F, Isodan, and Gluma resulted in the highest mortality values ($\geq$82%) for pulpal fibroblasts exposed to these products through 0.5-mm and 1-mm dentin discs.

DISCUSSION

Many products with different components and mechanisms of action are available on the dental market for the treatment of DH. Considering the way in which they are applied to overcome sensitization, not only the gingival tissues but also the dental pulp, via the dentinal tubules, are almost exposed to DDPs. It is known that DDPs can contain toxic components, such as glutaraldehyde, HEMA, triclosan, methacrylate resins, and sodium fluoride.[24,26,40] Therefore, the cytotoxicity of DDPs to delicate tissues is a significant issue, and their biocompatibility has been the subject of *in vitro* cytotoxicity studies.[33,36,41-43]

For this purpose, primary human periodontal ligament and pulp fibroblast cells, gingival fibroblasts, and odontoblast-like cells, as well as permanent cell lines, such as 3T3 and L-929, and primary and immortalized bovine dental papilla–derived cell lines have been used.[23,27,32-36]

It has been reported that primary cell lines are more suitable for *in vivo* conditions as a result of their specific metabolic activities.[44] The International Organization for Standardization, which provides guidance for the evaluation of the *in vitro* cytotoxicity of dental materials, reported that primary cell lines obtained from live cells can be used on occasions in which specific sensitivity should be determined.[45]

The nature of the testing methods has varied in that cytotoxicity is assessed either by exposing cells directly or indirectly to the test materials.[46,47] In the present study, the cytotoxic effects of DDPs were evaluated on primary gingival fibroblasts by direct contact testing because the gingiva was exposed to these products during improper application of the products.

In contrast, cytotoxicity to primary pulp fibroblasts was tested by the indirect contact method using dentin discs because dentin, as a result of its tubules, can act as a channel for the components released from a variety of restorative dental materials to reach the pulp tissue.[48] For this reason, dentin discs with different thicknesses were interpositioned between pulpal fibroblast cells and products to simulate clinical conditions. It was reported that 0.5 mm in thickness was optimal for evaluating a range of cytotoxic concentrations,[43] and a dentin barrier that was greater than 0.5 mm in thickness could dramatically reduce dentin permeability.[49] Therefore, in this study dentin discs with thicknesses of 0.5 mm and 1 mm were used.

In this study, the cytotoxicity of DDPs on gingival and pulpal fibroblasts was evaluated by applying the MTT assay. The MTT assay is a commonly used cell viability assay in evaluating the cytotoxicity of dental materials because of its ease of use and high sensitivity.[23,31,45,50,51] The MP value was determined using OD values as a result of the MTT assay.[39]

According to the results of this study, when different dilutions of components released from DDPs were tested on gingival fibroblast cells, Colgate Sensitive Pro-Relief, Clinpro White Varnish, and Topex were less cytotoxic than the other DDPs. In contrast, Systemp Desensitizer, Aqua-Prep F, Isodan, and Gluma were found to be more cytotoxic to both gingival and pulpal fibroblast cells.

It has been reported[24-26] that the cytotoxicity of a material is related to its composition. An example of

this relationship is HEMA, which is a low–molecular weight hydrophilic monomer that can be released from resin-based materials and penetrate the dentin tissue easily, affecting odontoblast vitality and physiological activity.[52-54] The low dose and long-term use of HEMA inhibits its inflammatory response ability. However, the cytotoxicity of HEMA was reported[55] to depend on time and concentration. Aqua-Prep F, Isodan, and Gluma are known to contain HEMA at rates of 10%-30%, 0%-40%, and 35%, respectively. Thus, the toxicity of Aqua-Prep F, Isodan, and Gluma detected in the present study might be related to their HEMA content.

Furthermore, Aqua-Prep F and Isodan contain sodium fluoride as well as HEMA. Sodium fluoride has been shown to be cytotoxic in low -pH environments.[56,57] Isodan has a low pH (2.2). In high concentrations, sodium fluoride prevented the proliferation of human epithelial tissue cells (HaCaT cells), and the cell reaction to sodium fluoride depends on the cell type.[58,59] Just as the cytotoxicity of Aqua-Prep F and Isodan might be related to HEMA and sodium fluoride, the combined effect of these two materials can also affect cytotoxicity. Topex and Clinpro White Varnish contain higher proportions of sodium fluoride than do Aqua-Prep F and Isodan. However, Topex and Clinpro White Varnish were cytotoxic only in high concentrations (50%), and these groups were not found to be cytotoxic in further dilutions. Therefore, the higher cytotoxicity of Aqua-Prep F and Isodan at all concentrations might be more closely related to HEMA than to sodium fluoride.

In contrast, Gluma, Smart Protect, and Systemp Desensitizer contain glutaraldehyde, which is used as a disinfectant and sterilizing agent against bacteria and viruses (2% solution). Glutaraldehyde causes coagulation of the plasma proteins in tubule fluid, resulting in a reduction in dentinal permeability[60]; thus, glutaraldehyde is added to DDPs and to dentin bonding agents for its desensitizing effects.[16] Therefore, the cytotoxicity of Gluma, Smart Protect, and Systemp Desensitizer found in this study might be related to their glutaraldehyde content, which is toxic to cells.

Similarly, the cytotoxicity of Systemp Desensitizer might be due to its ingredients, which include methacrylated monomers and glutaraldehyde, because methacrylated monomers may cause membrane lipid dissolution on cell membranes.[31,50]

Smart Protect contains triclosan, in addition to glutaraldehyde. Triclosan has been added to the active ingredients of some mouth rinses and dentifrices to prevent and treat gingivitis and plaque.[61] It was reported that triclosan induced cell death by apoptosis and by slowing the growth kinetics of the Smulow-Glickman (S-G) human gingival epithelial cell line,[62] and it was also reported that triclosan inhibited the adipocyte differentiation of human mesenchymal stem cells at high concentrations.[63] The cytotoxicity of Smart Protect could be attributed to glutaraldehyde and triclosan or to the combined effect of these and other ingredients.

Seal & Protect contains triclosan, like Smart Protect, as well as toxic components, such as di- and trimethacrylate resins and pentaacryloyldipentaerythrytol, phosphoric acid, functionalized amorphous silica, photoinitiators, and butylated hydroxytoluene. The ingredients of Seal & Protect have different rates of toxicity.[27,36,64,65] The cytotoxicity of Seal & Protect might be related to its ingredients, or it could be due to the interactions of all its contents.

Furthermore, Seal & Protect is clinically polymerized with blue light. Polymerization of methacrylate resins can reduce the cytotoxicity of resin monomers.[66] However, the application of blue light causes the formation of free radicals in cells and negatively affects cell division, causing oxidative stress in different cell series, as well as DNA modifications.[67-69] In this study, with the aim of eliminating the negative effects of blue light on polymerization, Seal & Protect was not polymerized with light. Therefore, the possible effects of blue light on the cytotoxicity of Seal & Protect could not be determined under these testing conditions.

BisBlock contains less than 5% oxalic acid, and D/Sense Crystal contains 2.5% nitric acid and 2.5% potassium dioxalate. Oxalic acid forms soluble salts with sodium, potassium, and ammonium ions and insoluble salts with calcium, magnesium, and iron ions.[70,71] In neutral and alkaline environments, calcium and oxalate can bind together, forming different-shaped crystals of calcium oxalate.[70] Topical usage of potassium oxalate results in the deposition of calcium oxalate crystals on the dentin surface. Oxalate reacts with the calcium compounds in dentin and promotes deposition of potassium oxalate. The precipitation of oxalic acid, as calcium oxalate monohydrate, was reported to be an intracellular toxin to normal human proximal tubule cells by inhibition of mitochondrial respiratory function in proximal tubular cells,[72] and it was also reported that the cytotoxicity of oxalate might be due to plasma membrane damage and organelle injury.[73]

The cytotoxicity of D/Sense Crystal could depend on potassium dioxalate, and the cytotoxicity of BisBlock might result from its oxalic acid content. The higher MP value of D/Sense Crystal, compared to that of BisBlock, on pulpal fibroblasts could be related to its ingredients and to the interactions of these ingredients with each other.

In addition, the manufacturers recommend etching the exposed tooth surface for 15 seconds with 32% phosphoric acid before applying BisBlock in clinical practice. In this study, phosphoric acid was not applied because of its probable toxic effects on fibroblast cells in addition to BisBlock.[65] So the application of 32% phosphoric acid could cause the cytotoxic effects of BisBlock in cases of its misapplication to gingival tissues.

UltraEZ contains 3% potassium nitrate and 0.25% sodium fluoride. An experimental mouth rinse, which contains 7% potassium nitrate and 0.025% sodium fluoride, similar to UltraEZ, was reported to be noncytotoxic to human esophageal squamous cell carcinoma (SCC) cells .[74] In the current study, UltraEZ was cytotoxic at 50% and 20% dilutions, and its cytotoxicity decreased to a considerable extent as the concentration decreased.

Colgate Sensitive Pro-Relief was the most recently introduced of the desensitizing products tested in this study. It contains L-arginine, calcium carbonate, glycerin, water, bicarbonate, hydrated silicate, and sodium saccharine. The essential component of the product is arginine, an amino acid, which is positively charged at a physical pH of 5-7.5 and contains a bicarbonate buffer and calcium carbonate. Colgate Sensitive Pro-Relief only showed cytotoxic effects in 50% dilutions, such as Topex and Clinpro White Varnish. It was reported that Colgate Sensitive Pro-Relief occluded exposed dentinal tubules and tubule plugs containing calcium and phosphate, and these products reached a depth of 2 μm into the dentinal tubules.[20,75]

When cytotoxicity was evaluated using different-thickness dentin discs, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were not cytotoxic. In addition, BisBlock, UltraEZ, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were found to be less cytotoxic to pulp fibroblasts, compared to the other DDPs. This finding can be explained by the dentinal tubule-occlusive effects of these products, in addition to their compositions.

The cytotoxicity of the DDPs in the current study might lack any clinical significance because of the buffering capacity of dentin and saliva.[76,77] Saliva can dilute the DDPs to some extent, and it can wash the tooth surfaces and gingiva. As the gingival surface is protected by highly keratinized epithelia cells, it is possible that cytotoxicity of the DDPs on the gingival surface might decrease clinically.

In addition, in this *in vitro* study, pulpal clearance and pulpal pressure were not simulated. In addition, the possible consequences of adding a large percentage product solution to the culture media (eg, change of pH and/or osmolality) were not determined in this study. Whether or not this practice can cause significant adverse effects on fibroblast cells could be investigated in future studies. Another important point is that the varied application methods might affect the cytotoxicity of different desensitizers as well (eg, blue light application after applying desensitizers or etching the exposed tooth surface with phosphoric acid before applying desensitizers in clinical practice). Furthermore, the DDPs included more than one cytotoxic component. The cytotoxic effects of the interactions between the components of these products should be investigated under *in vitro* and *in vivo* conditions.

CONCLUSION

Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were found to be less cytotoxic than the other DDPs used in this study. Further studies are necessary to simulate salivary components and their interactions with these products, as well as the effects of pulpal pressure and clearance on the cytotoxicity of DDPs.

Human Subjects Statement

This study was conducted in accordance with all the provisions of the local human subject oversight committee guidelines and policies. The approval code for this study is 2010/82. This study was conducted at Karadeniz Technical University.

Conflict of Interest

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

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Cuspal Flexure and Extent of Cure of a Bulk-fill Flowable Base Composite

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Clinical Relevance

Bulk-fill composites are an attractive alternative to reduce restoration placement time if sufficient cure can be ensured without an increase in shrinkage stress. This study provides evidence that supports bulk filling techniques and may help clinicians use this composite type without jeopardizing cure.

SUMMARY

Objectives: To investigate a bulk-fill flowable base composite (Surefil SDR Flow) in terms of cuspal flexure and cure when used in incremental or bulk techniques.

Methods: Mesio-occluso-distal cavities (4 mm deep, 4 mm wide) were prepared in 24 extracted molars. The slot-shaped cavities were etched, bonded, and restored in 1) two 2-mm increments Esthet-X HD (control), 2) two 2-mm increments Surefil SDR Flow, or 3) 4-mm bulk Surefil SDR Flow (N=8). The teeth were digitized after preparation (baseline) and restoration and were precisely aligned to calculate cuspal flexure. Restored teeth were placed in fuchsin dye for 16 hours to determine occlusal bond integrity from dye penetration. Extent of cure was assessed by hardness at 0.5-mm increments through the restoration depth. Results were analyzed with analysis of variance and Student-Newman-Keuls post hoc tests (α=0.05).

Results: Surefil SDR Flow, either incrementally or bulk filled, demonstrated significantly less cuspal flexure than Esthet-X HD. Dye penetration was less than 3% of cavity wall height and was not statistically different among groups. The hardness of Surefil SDR Flow did not change throughout the depth for both incrementally and bulk filled restora-

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tions; the hardness of Esthet-X HD was statistically significantly lower at the bottom of each increment than at the top.

Conclusions: Filling in bulk or increments made no significant difference in marginal bond quality or cuspal flexure for the bulk-fill composite. However, the bulk-fill composite caused less cuspal flexure than the incrementally placed conventional composite. The bulk-fill composite cured all the way through (4 mm), whereas the conventional composite had lower cure at the bottom of each increment.

INTRODUCTION

Visible light-activated (light-cured) composites are the material of choice for direct restorations because they offer prolonged manipulation time and on-command curing.[1] However, this desirable property has some drawbacks because the depth of light penetration is limited. To ensure adequate polymerization, light-activated composites must therefore be placed and cured in increments that are no more than 1.5 to 2 mm thick.[1-3] The need for curing composite restorations in multiple increments can make restorative procedures time consuming. Over the course of one year, an estimated 20 to 40 hours of chair time is used for light-curing.[3] Recently, a new generation of composites has been introduced that can be cured to depths of 4 mm or more[4-6] without compromising margin quality.[7-10] Cavities can now be filled and light-activated in one increment (bulk), simplifying the restorative procedure and saving time for the patient and clinician.

However, although the time-saving potential of this new class of composites is attractive, there was another major reason why filling in bulk has been discouraged. In dentistry, there has been a strong belief that incremental techniques should be used to reduce polymerization shrinkage stress.[1,11] Although this concept has been challenged by studies showing that incremental placement causes incremental tooth deformation that may lead to higher instead of lower stress,[12-14] incremental placement is still widely believed to alleviate shrinkage stresses. Although the relevance of incremental placement for stress reduction may remain a contentious topic,[15] it is important to keep in mind that many other factors are involved in the eventual level of shrinkage stresses that are likely more critical.[16] To take all relevant factors into account, cure and stress development should preferably be evaluated within the context of their application: as a restoration in a tooth.[17]

The objective of the current study was to evaluate if bulk placement of a bulk-fill flowable base composite is acceptable by examining three factors that are critical for initial clinical success: extent of cure, cuspal flexure, and bond integrity. The extent of cure is critical because adequate polymerization is a prerequisite for achieving optimal mechanical properties and biocompatibility, cuspal flexure is critical because it reflects the level of internal shrinkage stresses, and bond integrity is critical because of its role in structural reinforcement and sealing performance of a restoration.

METHODS AND MATERIALS

Specimen Preparation and Scanning

For this study (exempt approved by the institutional review board), 24 extracted human molars kept in 10% formalin acetate were selected in eight sets of three teeth that matched in shape and size. The sample size was based on previously published studies with similar methods; a sample size of eight has 95% confidence to detect a standard deviation difference of 0.7 between groups. The teeth were secured by their roots in stainless steel rings, pumiced to remove residual biofilms, and etched with 37% phosphoric acid solution to achieve a matte surface suitable for optical scanning. During the experiment, all teeth were kept hydrated in water, except during scanning.

Large mesio-occluso-distal slot-shaped cavities were prepared with a 245 carbide bur in a high-speed handpiece under copious amounts of water. During preparation, cavity width was checked with a digital caliper and cavity depth with a periodontal probe. After sectioning, later in the process, the cavity and cuspal dimensions were measured with image analysis software under a stereomicroscope (see the "Dye Penetration" section). The buccolingual width was 4.23 ± 0.13 mm and depth was 3.80 ± 0.32 mm (mean±standard deviation). Widths and depths were not statistically significant different among the three groups (analysis of variance [ANOVA], $p=0.9373$ and $p=0.5798$, respectively). The resulting cuspal widths were also not significantly different among buccal and lingual sides (ANOVA, $p=0.5452$) or between groups (ANOVA, $p=0.6413$). About 10 to 15 minutes after preparation, the prepared teeth, along with reference spheres embedded in the steel rings, were digitized with an optical scanner (Comet xS 3D Optical Digitizing System, Steinbichler Vision Systems, Neubeuern, Germany). Each point on the scanned surfaces was measured with 5-μm accuracy; the measured points

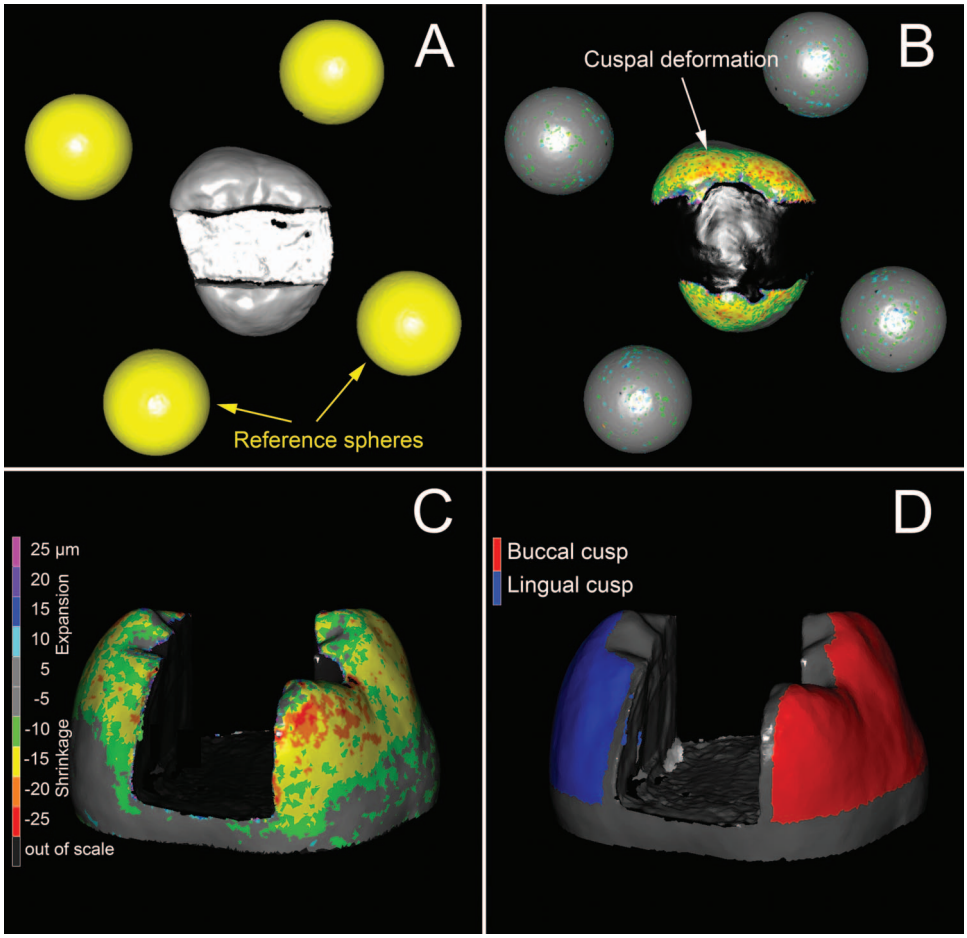

Figure 1. *The preparation scan (A) and restoration scan (B) were aligned in Cumulus software using four stainless steel spheres as the unchanged reference areas shown in yellow (A). Deformation of the buccal and lingual surfaces, that is, cuspal flexure, is visualized in a linear color scale (B, C). Buccal and lingual cuspal surfaces were selected (D) for the calculation of cuspal flexure.*

were 60 µm apart (resolution). The scans of the prepared teeth were used as the baseline (Figure 1A).

Restorative Procedures

Materials used in the study and their compositions are shown in Table 1. A total etch (etch and rinse) bonding technique was used. The cavities were etched with 34% phosphoric acid gel (Dentsply Caulk, Milford, DE, USA) for 15 seconds, rinsed with water, and blotted dry using Kimwipes (Kimberly-Clark, Roswell, GA, USA). Prime & Bond Elect (Dentsply Caulk) was applied and agitated for 20 seconds and gently air-dried for 5 seconds. The mesial and distal halves of the preparation were light-cured for 10 seconds each (VIP Junior, Bisco, Schaumburg, IL, USA). The light intensity was 600 mW/cm^2, measured with a radiometer (Model 100, Demetron Research Corp, Danbury, CT, USA) periodically during the experiments. The three matched teeth from each set were randomly assigned to be restored as follows: 1) two 2-mm horizontal increments using Esthet X HD (Dentsply Caulk) as a control; 2) two 2-mm horizontal increments using Surefil SDR Flow (Dentsply Caulk); and 3) one 4-mm bulk-filling using Surefil SDR Flow. The mesial and distal halves of each increment or bulk material were light-cured for 20 seconds each from the occlusal direction. The composite surfaces were wiped with an alcohol pad to remove the oxygen inhibition layer. After 20 minutes in water, the restored teeth were digitized with the optical scanner.

Cuspal Flexure Analysis

The baseline and restoration scans were accurately aligned by fitting the stainless steel spheres as unchanged references (Figure 1A) using Cumulus software (Copyright Regents of the University of Minnesota).[18] The amount of cuspal flexure was calculated using a custom-developed CuspFlex software. Flexure was determined for the buccal and lingual surfaces from the area between the cusp ridges and the level of the pulpal floor (Figure 1C,D).

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

Material	Composition	Application
Esthet X HD High Definition Micro Matrix Restorative (A2-O) Lot 1301031	Barium boron fluoro-alumino- silicate glass <30% Barium boron alumino-silicate glass <50% Hydrophobic amorphous fumed silica <5% Silica (amorphous) <5% Urethane modified bisphenol A glycidyl methacrylate dimethacrylate <10% Polymerizable dimethacrylate resins <20% Colorants: inorganic iron oxides and titanium dioxide	Light cure 20 s
SureFil SDR Flow – Posterior Bulk Fill Flowable Base (UNIV) Lot 1208221	Barium boron fluoro-alumino-silicate glass <50% Silicon dioxide (amorphous) <5% Strontium aluminosilicate glass <50% Titanium dioxide <1% Polymerizable dimethacrylate resins <10% Polymerizable urethane dimethacrylate <25% Colorants: synthetic inorganic iron oxides	Light cure 20 s
Caulk 34% Tooth Conditioner Gel Lot 121120	Phosphoric acid 34% Blue colorant: fluorescent organic dye	Etch 15 s, rinse with water 10 s, blot dry
Prime&Bond Elect Universal Dental Adhesive Lot 121120	Mono-, di-, and trimethacrylate resins PENTA (dipentaerythritol penta acrylate monophosphate) Diketone Organic phosphine oxide Stabilizers Cetylamine hydrofluoride Acetone Water	Apply and agitate 20 s using microbrush applicator tip, gently air dry 5 s, light cure 10 s

[a] *All materials are from Dentsply Caulk, Milford, DE, USA.*

The average deflection of each surface (buccal or lingual) was calculated from the notional volume change (integrated difference across the selected areas) divided by the selected surface area. The cuspal flexure was the sum of buccal and lingual cuspal deflections.

Dye Penetration

Dye penetration of the occlusal interface was assessed to verify the bond integrity. After the restored teeth had been scanned, restoration margins were finished with composite finishing burs (LogicSet, Axis Dental Corp, Coppell, TX, USA). Root apices were obstructed with utility wax, and the roots were painted with nail polish. The teeth were then placed in 0.5% basic fuchsin dye solution overnight (16 hours), embedded, and sectioned buccolingually into two halves. One half was used for the bond integrity assessment and the other half for the depth of cure evaluation. For the bond evaluation, the samples were sectioned buccolingually into 1-mm slices, yielding 4-6 slices per sample. Each slice was imaged using a stereomicroscope with a charge coupled device camera (SZX16 & UC30, Olympus, Tokyo, Japan). The distances of dye leakage at the occlusal margins along the buccal and lingual interfaces were measured by two independent evaluators using image analysis software (Stream Basic, Olympus Soft Imaging Solution GmbH, Münster, Germany) (Figure 2). If the distances measured by the two evaluators differed more than 10%, a consensus value was reached. The dye penetration distances were averaged for each tooth. In addition, the wall height of each cavity interface was measured from the occlusal margin to the pulpal floor.

Depth of Cure Measurement

The other tooth half was used to assess the depth of cure by measuring hardness on the cross-sectional surface.[19,20] Thus, the hardness was measured one day after the restoration had been cured. The tooth halves were embedded in acrylic resin, and the cross-sectioned surfaces were serially polished with 400- and 600-grit silicon carbide paper followed by 1 μm and 0.05 μm alumina pastes. Vickers hardness (QV-1000 Micro Hardness Tester, Qualitest USA LC, Fort Lauderdale, FL, USA) was measured from indentations made across the restoration at 0.5 mm increments from the occlusal surface to the bottom of the restoration (pulpal floor) (Figure 3A). The indentation load was 200 g at 15 seconds dwell time.

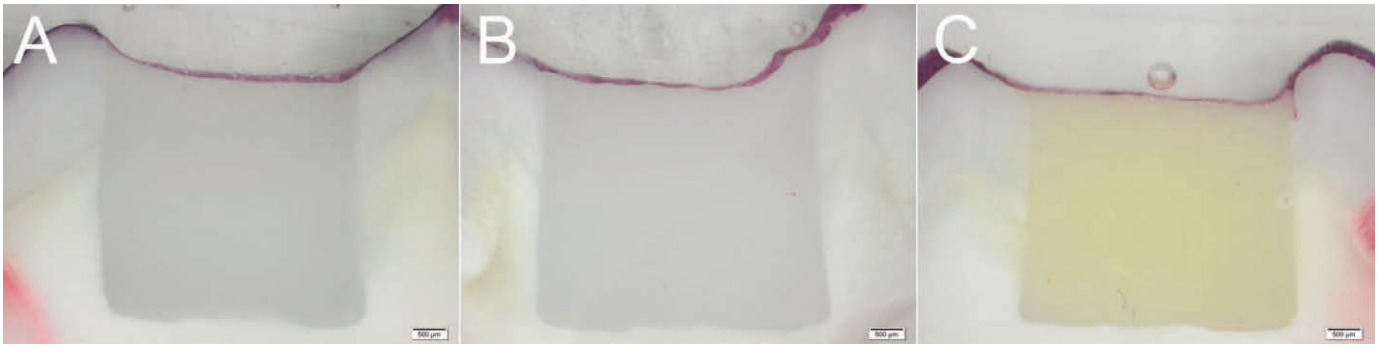

Figure 2. *Buccolingual sections of restored teeth illustrating no or small dye penetration at the occlusal enamel interface: (A) Surefil SDR Flow placed in bulk. (B) Surefil SDR Flow placed in two increments. (C) Esthet X HD placed in two increments.*

Statistical Analysis

The differences in cuspal flexure and dye penetration among groups as well as differences in hardness values among various depths within each group were statistically analyzed with ANOVA followed by Student-Newman-Keuls post-hoc tests (significance level 0.05).

RESULTS

Cuspal flexure and dye penetration distance are shown in Table 2. Surefil SDR Flow, either incrementally or bulk filled, caused significantly less cuspal flexure than Esthet X HD (ANOVA, $p=0.0211$, followed by Student-Newman-Keuls post-hoc test, $p<0.05$). Dye penetration distances at the occlusal enamel interface were less than 3% of the wall height and did not show statistically significant differences among groups (ANOVA, $p=0.0948$). Images of the buccolingual sections of restored teeth, one from each group, are shown in Figure 2; these show no or little dye penetration.

Vickers hardness numbers at various depths in the restorations of each group are shown in Figure 3B. Esthet X HD had higher hardness than Surefil SDR Flow. Incrementally cured Esthet X HD had the highest hardness values at the surface of each increment before gradually decreasing with increasing depth. The hardness value of Esthet X HD was significantly lower at the bottom of each increment

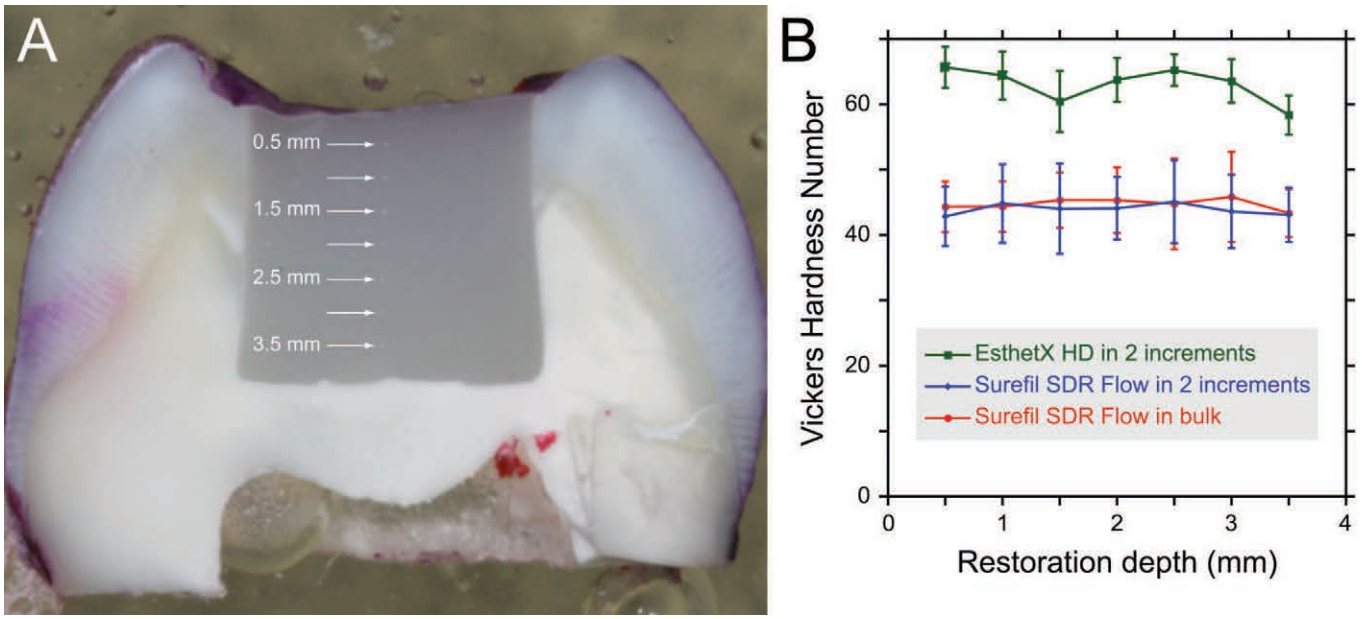

Figure 3. *(A) Cross-section of a restored tooth showing position of Vickers indentations. (B) Plots of Vickers hardness numbers at various depths in the restorations (extent of cure).*

Table 2: *Cuspal Flexure and Dye Penetration Distance (Mean± Standard deviation; microns)*[a]

	Esthet X HD Incremental	Surefil SDR Flow Incremental	Surefil SDR Flow Bulk
Cuspal flexure (μm)	22.8 ± 3.9 a	17.2 ± 4.2 b	18.9 ± 2.0 b
Dye penetration (μm)	85 ± 27 a	60 ± 32 a	47 ± 33 a

[a] *Different letters indicate statistical differences between groups (analysis of variance followed by Student-Newman-Keuls post hoc tests, significance level 0.05).*

compared with values closer to the top of increments (ANOVA, $p=0.0020$, followed by Student-Newman-Keuls post-hoc test, $p<0.05$). The hardness values of incremental or bulk-filled Surefil SDR Flow were not significantly different at any depth (Student-Newman-Keuls post-hoc test, $p<0.05$) and did not significantly change throughout the 4-mm depth of the restorations (ANOVA, incremental $p=0.9881$ and bulk-filled $p=0.9786$).

DISCUSSION

Bulk filling has long been viewed with skepticism, but the advent of viable bulk-fill composites that promise to simplify and shorten restoration procedures may be changing this perception. The effect of bulk filling on cure and stress in a composite restoration cannot be easily determined clinically nor can it be extrapolated from the material properties alone. To evaluate the viability of a bulk-fill composite, this study examined three factors in *in vitro* restored teeth: cuspal flexure, extent of cure, and marginal bond.

Three Factors

The three selected factors could be considered most affected by a bulk fill technique. Of the three, the extent of cure is obvious because it determines the feasibility for bulk cure. Cure also affects polymerization shrinkage stresses. Stress is a local condition that is calculated by taking into account the properties of the restored tooth, transient properties of the composite, shapes of the tooth-restoration complex, and the state of bonding. In this study stresses were not determined because that required complex calculations.[21] Instead, cuspal flexure was determined.[22-24] Cuspal flexure is caused by stresses, and thus inherently encompasses all attributes involved in the shrinkage stress, including the transient composite properties.[17,25] Marginal bond, the third factor, was examined because shrinkage stress is generated when composite polymerization contraction is constrained by the bonding to the tooth structure. The state of the restoration bonding must therefore be considered when assessing shrinkage stresses.

Cuspal Flexure

The cuspal flexure for the bulk-fill flowable composite used in this study did not show a significant difference between the incremental or bulk-filling techniques. The cuspal flexure with the bulk-fill flowable base composite restorations was significantly lower than the conventional nonflowable restorative composite that was used as a reference. This suggests that the new bulk-fill flowable base composite generated lower shrinkage stresses in the tooth than those of a conventional, well-accepted restorative composite system.[5,26] It also suggests that for controlling shrinkage stresses within a clinical context (ie, restoration of a tooth), the difference between incremental or bulk-fill techniques is probably marginal.[13,20]

Extent of Cure

Interpretation of cuspal flexure results requires caution. As noted previously, differences in cure also cause differences in flexure. For example, inadequate cure leads to lower elastic modulus and less polymerization contraction, which results in less cuspal flexure and hence lower shrinkage stresses in a restored tooth. The trade-off between less cure for lower shrinkage stress is undesirable, and therefore obtaining lower cuspal flexure due to less cure is not necessarily a favorable outcome. To ensure that the cure was adequate or comparable, the extent of cure was determined throughout the depth of the restorations using hardness measurements.

The hardness measurements of the bulk-fill flowable base composite indicated that a homogeneous cure through the entire 4-mm depth of the composite was achieved with both the incremental and bulk cure. If the cure at the surface is assumed to have been adequate, the cure throughout the 4-mm-deep restorations must have been adequate also, ensuring optimal mechanical properties, biocompatibility, and chemical stability regardless of whether incremental or bulk curing techniques were used. Unlike the bulk-fill composite, the conventional composite showed a decrease in hardness (and thus extent of cure) at the bottom of each 2-mm increment, demonstrating that composites that are not designed for bulk curing should be cured in increments to avoid compromising their mechanical and biophysical properties. It is important to mention that no direct comparisons were made

about the degree of cure of the bulk-fill and conventional composites using the hardness data because hardness is a material-specific property that also depends on composition (eg, filler distribution, resin type).[19] The lower hardness values of the bulk-fill composite, consistent with previous studies,[26] were likely due to the lower filler loading of this flowable restorative.[4]

Marginal Bond

The third factor that was examined was the state of the bond at the occlusal margins. Clinically, good bonding between composite and tooth structure is critical for restoring the structural integrity of a restored tooth[27] and for sealing the inside of the tooth structure from bacteria and their nourishments. A restoration bond is challenged by polymerization shrinkage as well as stresses caused by thermal and mechanical loading. Resisting those challenges requires sufficient bond strength. Although strength or longevity of the bond are important for the performance of a restoration, it was not the objective of this study. For the present study design, intact bonding needed to be verified to ensure that the measured cuspal flexure was due to polymerization shrinkage and not modified by bond failure. Debonding would relieve shrinkage stresses and reduce the cuspal flexure, wrongly suggesting lower shrinkage stresses. Clinically, reduction in shrinkage stress due to debonding is also undesirable. Penetration of the fuchsin dye revealed that the bond remained largely intact for all groups, thus indicating comparable quality of the bonds among the three groups. Therefore, comparison of the cuspal flexure among the three groups was justified and could be used to draw conclusions about the effect of bulk filling on the stresses in restored teeth.

Interpretation of Cuspal Flexure

Various studies have confirmed the ability of bulk-fill composites to cure much deeper than conventional composites,[4-6] and *in vitro* cuspal flexure and marginal gap studies have not found compelling evidence for avoiding bulk-fill techniques.[7-10] Cuspal flexure measurements are a convenient technique for comparing the state of stresses in teeth restored with different composites or filling techniques, provided that the tooth properties, shapes, and bonding conditions are comparable.[21] However, the performance of a restoration is not determined by shrinkage stress. Stress cannot be extrapolated to performance without the context of strength. A weak interface will fail at low stress values, and thus a low shrinkage stress state can still have little relevance for clinical performance. Moreover, polymerization shrinkage stress is a temporary condition because it is likely to be compensated by hygroscopic expansion and stress relaxation.[28-30] If a bond survives the initial shrinkage stresses, other properties may turn out to be more important for the life expectancy of a restoration.

Based on these considerations and the results of this study, it can be postulated that the most important aspect of a composite restoration technique is maintaining acceptable cure and intact interfaces. The choice between an incremental or bulk-filling technique should be guided by those requirements, rather than by shrinkage stress considerations. From cross-sectioned samples, we observed that the low-viscosity flowable composite provided better adaptation to the cavity walls, floor, and between composite layers in the incremental fillings than the conventional composite. The lower microhardness of the flowable composite compared with the conventional composite supports the recommendation that under clinical conditions this flowable material should be used as a base/liner or dentin replacement.[31] The potential to attain adequate polymerization in deep layers offered by the new generation of bulk-fill composites is an advantage in critical areas where curing can be compromised, such as in the gingival portion of a proximal restoration where a bulk-fill flowable base composite like Surefil SDR Flow is recommended under a universal or posterior composite.

CONCLUSIONS

1. Cuspal flexure was lower with the bulk-fill flowable base composite than with an incrementally cured conventional composite.
2. Filling in bulk or increments made no significant difference in the cuspal flexure of a bulk-fill flowable base composite.
3. The bulk-fill flowable base composite restoration cured all the way through (4 mm) irrespective of bulk or incremental cure, whereas the cure of the conventional incrementally cured composite decreased toward the bottom of each (2 mm) increment.
4. Clinicians can use the bulk-filling technique with the tested bulk-fill composite without jeopardizing depth of cure or elevating the polymerization shrinkage stresses.

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Human Subjects Statement

This study was conducted in accordance with all the provisions of the local human subjects' oversight committee guidelines and policies. The approval code for this study is #13-02372-XM. This study was conducted at the College of Dentistry, University of Tennessee Health Science Center.

Conflict of Interest

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

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Microshear Bond Strength of Resin Cements to Lithium Disilicate Substrates as a Function of Surface Preparation

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Clinical Relevance

There is an urgent need to establish a reliable protocol to treat the intaglio surface of lithium disilicate glass-ceramic, as several cementation protocols are currently used, including self-adhesive cements.

SUMMARY

Objectives: To investigate the effect of hydrofluoric acid (HF) etching, silane solution, and adhesive system application on the microshear bond strength (μSBS) of lithium disilicate glass-ceramic (LD) to three resin cements.

Materials and Methods: Circular bonding areas were delimited on the lithium disilicate surfaces using a perforated adhesive tape. Specimens were assigned to 18 subgroups (n=12) according to surface treatment: NT = no treatment; HF = 4.8% HF for 20 seconds; silane solution: (1) no silane; (2) Monobond Plus, a silane/10-methacryloyloxydecyl dihydrogen phosphate solution for 60 seconds; (3) Monobond Plus+ExciTE F DSC, a dual-cure adhesive; and resin cement: (1) Variolink II, a bisphenol A diglycidyl ether dimethacrylate (bis-GMA)–based, hand-mixed, dual-cure resin cement; (2) Multilink Automix, a bis-GMA–based, auto-mixed, dual-cure resin cement; (3) RelyX Unicem 2, a self-adhesive, auto-mixed, dual-cure resin cement. Tygon tubes (Ø=0.8 mm) were used as cylinder matrices for resin cement application. After 24 hours of water storage, the specimens were submitted to the μSBS test. Mode of failure was evaluated under an optical microscope and classified as adhesive, mixed, cohesive in resin cement, or cohesive in ceramic. Data were statistically

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analyzed with three-way analysis of variance and Dunnett test ($p<0.05$).

Results: When means were pooled for the factor surface treatment, HF resulted in a significantly higher μSBS than did NT ($p<0.0001$). Regarding the use of a silane solution, the mean μSBS values obtained with Monobond Plus and Monobond Plus+ExciTE F DSC were not significantly different but were higher than those obtained with no silane ($p<0.001$). Considering the factor resin cement, Variolink II resulted in a significantly higher mean μSBS than did RelyX Unicem 2 ($p<0.03$). The mean μSBS for Multilink Automix was not significantly different from those of Variolink II and RelyX Unicem 2. According to Dunnett post hoc test ($p<0.05$), there was no significant difference in μSBS between the different resin cements for HF-etched and silanized (with or without adhesive application) LD surfaces.

Conclusion: LD may benefit from pretreatment of the inner surface with HF and silanization, regardless of the resin cement used.

INTRODUCTION

Following the demand for tooth-colored, high-strength restorations, ceramic systems have been developed with different proportions of glassy and crystalline phases to improve their mechanical properties while maintaining good esthetic properties. Among the options currently available is lithium disilicate glass-ceramic (LD), which was commercially marketed in 1998 by Ivoclar Vivadent as IPS Empress 2 and fabricated using a lost-wax and heat-pressed technique.[1] The material was later reformulated and relaunched as ingots that can be press-fit (IPS e.max Press, Ivoclar Vivadent, Schaan, Liechtenstein) or as CAD/CAM blocks (IPS e.max CAD, Ivoclar Vivadent) that can be milled to fabricate monolithic restorations (anterior or posterior crowns, implant crowns, inlays, onlays, or veneers).[2]

Unsupported glass ceramics—even those with high flexural strength—are prone to fracture under chewing loads, which justifies the use of adhesive cementation.[3,4] Hydrofluoric acid (HF) etching is commonly indicated to partially dissolve the glassy phase and to create microporosities. The HF exposure time depends on the ceramic microstructure and on the HF concentration, which usually ranges between 2.5% and 10%.[5] The manufacturer of LD specifically recommends etching the intaglio surface with 4.8% HF for 20 seconds. The crystals are exposed and serve as retentive surfaces for resin cement interlocking, which enhances adhesion. Furthermore, the application of a coupling agent (silane) onto the pre-etched intaglio surface is required. This bifunctional adhesion enhancer creates a chemical interaction between the silica in the glassy phase of the ceramic and the methacrylate groups of the resin through siloxane bonds.[6] After etching and silanization, there is an increase in surface energy and wettability, decreasing the contact angle between the ceramic and resin cement.[7] However, HF is a hazardous substance, and thus a simplified, safe, and faster conditioning process would be desirable.

Depending on the indication, the dentist has to select resin-based cements with different bonding strategies. Resin cements are recommended for adhesive cementation because of their low solubility in the oral environment, reduced microleakage at the restoration-tooth interface, good optical properties, and low incidence of marginal staining and recurrent caries.[8] Self-adhesive cements represent the newest and most simplified category of resin cements, which rapidly gained popularity to lute all-ceramic restorations as a result of their ease of use. In fact, the pretreatment of the substrate with etchant and/or adhesive is not recommended with self-adhesive resin cements.[9] Nonetheless, most dentists are still confused regarding the bonding protocols when using resin-based cements with LD restorations.[10] Bonding effectiveness may influence the long-term clinical success of all-ceramic restorations; thus, it seems reasonable to identify the most reliable and effective luting protocol at the cement/ceramic restoration interface. Since only scarce information is available with regard to the bond strength of simplified resin cements to LD restorations, this laboratory research aimed at investigating the effect of HF etching, silane solution, and adhesive application on the microshear bond strength (μSBS) of LD to dual-cure resin cements representing three strategies: hand-mixed bisphenol A diglycidyl ether dimethacrylate (bis-GMA)–based; auto-mixed bis-GMA–based; and auto-mixed self-adhesive. The null hypothesis tested was that the three factors used in this project would not influence mean μSBS.

METHODS AND MATERIALS

The materials and respective compositions are displayed in Table 1. Thirty-six IPS e.max CAD blocks (Ivoclar Vivadent) were cut into 72 rectangu-

Table 1: *Materials Used, Compositions, and Batch Numbers*

Material (Manufacturer)	Composition	Batch Number
IPS e.max CAD blocks (Ivoclar Vivadent)	Lithium disilicate glass-ceramic	S17323
IPS Ceramic Etching Gel (Ivoclar Vivadent)	4.8% hydrofluoric acid	S13497
Monobond Plus (Ivoclar Vivadent)	Ethanol, 3-trimethoxysilylpropyl methacrylate, methacrylated phosphoric acid ester (10-MDP), disulfide acrylate	S14727
ExciTE F DSC (Ivoclar Vivadent)	Dimethacrylates, HEMA, phosphonic acid acrylate, catalysts, stabilizers, fluoride, ethanol, silicon dioxide	S08275
Variolink II (Ivoclar Vivadent)	bis-GMA, UDMA, TEGDMA, ytterbium trifluoride, boroaluminofluorosilicate glass, spheroidal mixed oxide, benzoylperoxide, stabilizers, pigments	R69347
Multilink Automix (Ivoclar Vivadent)	Dimethacrylates, HEMA, t-amine (J05820), silicon dioxide filler, ytterbium trifluoride, catalysts, stabilizers, pigments, dibenzoyl peroxide	S14543
RelyX Unicem 2 (3M ESPE)	Methacrylate monomers containing phosphoric acid groups; methacrylate monomers, silanated fillers, alkaline (basic) fillers; initiator components, pigments, stabilizers, rheological additives	518863

Abbreviations: bis-GMA, bisphenol A diglycidyl methacrylate; DMA, aliphatic dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethyleneglycol dimethacrylate; UDMA, uretane dimethacrylate.

lar sections (16 mm×2 mm) using a low-speed diamond wheel saw (Model 650, South Bay Tech Inc, San Clemente, CA, USA) under water irrigation. After cleaning ultrasonically with distilled water for 15 minutes, LD specimens were fired following the crystallization program recommended by Ivoclar Vivadent. After cooling, specimens were positioned in polyvinyl chloride (PVC) plastic rings and embedded in epoxy resin (Epo-Thin Resin, Buehler Inc, Lake Buff, IL, USA) and wet polished with up to 600-grit silicon carbide paper for one minute. The delimitation of the bonding area was conducted in accordance with the method of Shimaoka and others[11] (Figure 1). An acid-resistant, double-sided adhesive tape (Scotch Permanent Double Sided Tape, 3M, St Paul, MN, USA) was perforated with three 0.8-mm–diameter holes and positioned over the ceramic surface. Eighteen groups (n=12) were created using the following combination of factors (a 2×3×3 design):

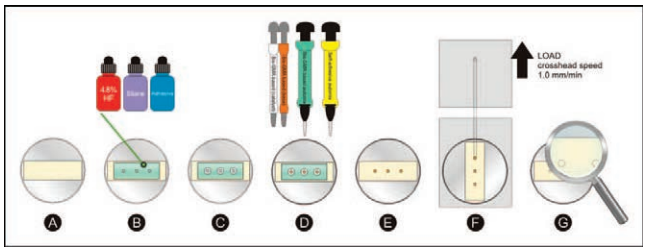


Figure 1. *Schematic overview of the microshear bond strength test (μSBS) setup. (A) LD block embedded in epoxy resin; (B) Adhesive application over the double-sided tape with three perforations (Ø=0.8 mm); (C) Tygon tubes coinciding with the pretreated surfaces; (D) Insertion of resin cements in the tubes; (E) Tubes and tapes were removed after 24 hours of water storage; (F) μSBS at a crosshead speed of 1.0 mm/min; (G) Failure analysis.*

- Surface treatment: 1) NT = no treatment; 2) HF = the ceramic surfaces were etched with 4.8% HF (IPS Ceramic Etching Gel, Ivoclar Vivadent) for 20 seconds, thoroughly rinsed with water spray, and ultrasonically cleaned in distilled water for 180 seconds.
- Silane solution: 1) No silane; 2) Monobond Plus (Ivoclar Vivadent), an ethanol-based silane coupling and 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) solution, was applied with a brush and allowed to react for 60 seconds. Subsequently, the excess was dispersed with a strong stream of air to ensure the solvent evaporation. 3) Monobond Plus+ExciTE F DSC (Ivoclar Vivadent), a dual-curing single-component adhesive, was applied for 10 seconds and air-dried for five seconds.
- Resin cement: 1) Variolink II (Ivoclar Vivadent): equal amounts of base and catalyst paste of a bis-GMA–based, hand-mixed, dual-cure resin cement were mixed carefully for 10 seconds. 2) Multilink Automix (Ivoclar Vivadent): a mixing tip was used to mix the base paste and catalyst of a bis-GMA–based, auto-mixed, dual-cure resin. 3) RelyX Unicem 2 (3M ESPE): a mixing tip was used to mix the base paste and catalyst of phosphate monomer–based, self-adhesive, auto-mixed, dual-cure resin cement.

After the surface treatment (NT or HF) and application of the silane solution (no silane, Mono-

bond Plus, or Monobond Plus+ExciTE F DSC), translucent Tygon tubes (Tygon Medical Tubing, Saint-Gobain, Akron, OH, USA) with an internal diameter of 0.8 mm and a height of 0.5 mm were used as matrices. Each tube was positioned over the tape, ensuring that the lumen coincided with the circular ceramic area exposed by the perforation. One trained operator using magnifying loupes (Optivisor, Donegan Optical Company, Lenexa, KS, USA) positioned the matrices on the ceramic surfaces; this was followed by careful insertion of the resin cement into each matrix. A Mylar strip was positioned over the filled tube and gently pressed in place. Resin cements were cured for 40 seconds using a LED light-curing unit (Translux Power Blue, Heraeus Kulzer, Hanau, Germany) with an output of 650 mW/cm^2. The intensity was checked daily. After 24 hours of storage in distilled water at 37°C, matrices and tapes were carefully removed using a sharp blade to expose the resin cement cylinders. Each specimen was examined using magnifying loupes to identify specimens containing possible defects in the resin cement cylinder (bubbles, flow of resin cement beyond the limits of the bonding area, and mismatch between the cylinders and their respective delimited area). The PVC rings were attached to a shear-testing jig. A thin wire (0.2-mm diameter) was looped around the base of each resin cement cylinder, in contact with half of its circumference, keeping the setup aligned to ensure the correct orientation of the shear forces. The cement/ceramic interface was then tested under shear mode in a universal testing machine (Instron 4444, Instron Corporation, Canton, MA, USA) at a crosshead speed of 1 mm/min until failure. The μSBS were calculated in MPa by dividing the load at failure by the surface area (mm^2) of each specimen. The results were statistically analyzed (IBM SPSS 21, IBM, Chicago, IL, USA) with three-way analysis of variance (ANOVA) and Dunnett multi-comparison post hoc test ($p<0.05$). The failure mode of debonded specimens was determined using 3.5× magnification and was classified as adhesive (A), mixed (M), cohesive in resin cement (CR), or ceramic (CC). Randomly selected specimens from each group were prepared for morphological examination under a field-emission scanning electron microscope (FESEM). Two extra LD specimens were prepared for morphological analysis of the HF-treated vs untreated surfaces. After drying at room temperature, the specimens were mounted on aluminum stubs with adhesive carbon tape (PELCO Carbon Conductive Tape, Ted Pella Inc, Redding, CA, USA) and colloidal quick-drying silver paint (PELCO Colloidal Silver, Ted Pella Inc). Sputter-coating was done with gold-palladium by means of a sputter-coater (Polaron E-5100 Sputter Coater, Polaron Equipment Ltd, Watford, UK) at 20 mA for 90 seconds. Specimens were observed under a FESEM (S-4700, Hitachi High Technologies America Inc, Pleasanton, CA, USA) at an accelerating voltage of 5.0 kV and a working distance of 12.0-13.0 mm.

RESULTS

The mean μSBS and fracture analyses are summarized in Table 2. No pretesting failures occurred. For the groups that did not receive HF etching or silane application (NT–no silane–Variolink II; NT–no silane–RelyX Unicem 2; and NT–no silane–Multilink Automix), mean μSBSs were close to zero and presented adhesive failures. When means were pooled for surface treatment, HF resulted in a significantly higher μSBS than did NT ($p<0.0001$). Regarding the silane solution, the mean μSBS values for Monobond Plus and for Monobond Plus+ExciTE F DSC were not significantly different; however, they were significantly higher than those of no silane ($p<0.001$). Considering the factor resin cement, Variolink II resulted in a significantly higher mean μSBS than did RelyX Unicem 2 ($p<0.03$). The mean μSBS values for Multilink Automix were not significantly different from those of Variolink II and RelyX Unicem 2. According to the Dunnett post hoc test ($p<0.05$), there were no significant differences in μSBS values between pairs of different resin cements for HF-etched + silanized (regardless of adhesive application) LD surfaces.

The morphology of nontreated LD after polishing (Figures 2A and 3A) showed a smooth surface without any retentive features. Etching with 4.8% HF for 20 seconds (Figures 2B and 3B) created a network of microporosities on the LD surface. The top view of etched LD in Figure 2B depicted an array of exposed LD crystals. Figure 4A shows a mixed failure for group NT+Monobond Plus+Multilink Automix, whereas Figure 4B is a higher magnification of the area of the resin cement fracture.

DISCUSSION

Although difficulties in bond strength testing and its interpretation are well-known,[12] there is still no alternative approach with which to test bond strength with similar efficiency in terms of time and cost. When conventional "macro" bond strength tests are used, a large number of cohesive failures are observed.[13] When the specimen size is de-

Table 2: *Mean Microshear Bond Strength (MPa±standard deviation [SD]) and Failure Mode (%)*

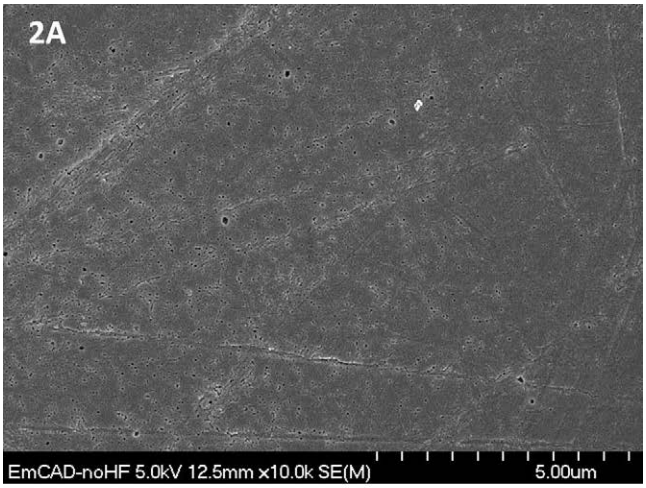
Surface Treatment[a]	Silane Solution[a]	Resin Cement[a]	Mean μSBS ± SD[b]	Failure Mode, %[c]
NT	No silane	Variolink II	0.28 ± 0.30 G	A = 100; M = 0; CR = 0; CC = 0
		Multilink Automix	0.18 ± 0.25 G	A = 100; M = 0; CR = 0; CC = 0
		RelyX Unicem 2	3.07 ± 1.07 F	A = 100; M = 0; CR = 0; CC = 0
	Monobond Plus	Variolink II	28.90 ± 6.20 BCDE	A = 66; M = 34; CR = 0; CC = 0
		Multilink Automix	27.70 ± 6.50 CDE	A = 75; M = 25; CR = 0; CC = 0
		RelyX Unicem 2	25.96 ± 5.46 DE	A = 66; M = 44; CR = 0; CC = 0
	Monobond Plus+ExciTE DSC	Variolink II	30.16 ± 5.98 BCDE	A = 0; M = 100; CR = 0; CC = 0
		Multilink Automix	24.64 ± 5.23 DE	A = 92; M = 8; CR = 0; CC = 0
		RelyX Unicem 2	21.34 ± 5.35 E	A = 34; M = 66; CR = 0; CC = 0
HF	No Silane	Variolink II	42.74 ± 10.38 AB	A = 50; M = 50; CR = 0; CC = 0
		Multilink Automix	28.76 ± 5.28 BCDE	A = 66; M = 34; CR = 0; CC = 0
		RelyX Unicem 2	22.68 ± 6.85 E	A = 34; M = 66; CR = 0; CC = 0
	Monobond Plus	Variolink II	40.70 ± 8.34 AB	A = 0; M = 100; CR = 0; CC = 0
		Multilink Automix	45.68 ± 9.65 A	A = 0; M = 100; CR = 0; CC = 0
		RelyX Unicem 2	41.78 ± 6.45 A	A = 0; M = 100; CR = 0; CC = 0
	Monobond Plus+ExciTE DSC	Variolink II	42.46 ± 5.08 A	A = 0; M = 100; CR = 0; CC = 0
		Multilink Automix	39.87 ± 9.16 ABC	A = 0; M = 100; CR = 0; CC = 0
		RelyX Unicem 2	35.86 ± 8.05 ABCD	A = 0; M = 100; CR = 0; CC = 0

Abbreviation: SD, standard deviation; μSBS, microshear bond strength.
[a] *NT, no treatment; HF, 4.8% hydrofluoric acid.*
[b] *Means with the same small capital letter are not significantly different (Dunnett test, $p<0.05$).*
[c] *A, adhesive; M, mixed; CR, cohesive in resin cement; CC, cohesive in ceramic.*

creased the probability of such flaws occurring is reduced, which favors the utilization of the micromechanical approach. Considering the 216 specimens tested in this study, no cohesive failures (either in resin cement or in ceramic) were observed. The circular bonding area delimitation may have induced better stress distribution around the adhesive interface and prevented the fracture from occurring beyond the limits of the cylinder.[14] Moreover, since the experimental setup did not require any specimen cutting or trimming, pretesting failures were avoided.

Regarding the clinical relevance of testing conventional bis-GMA–based resin cements vs a self-adhesive resin cement, clinical trials are not abundant in the literature. A recent systematic review[15] evaluated 12 clinical studies and indicated 97.8% as the cumulative short-term survival rate (up to five years) of 696 LD single crowns. However, the evidence for medium-term survival rate (five to 10 years) of 96.7% is still limited to just two studies. Regarding 145 LD fixed dental prostheses, five-year and 10-year cumulative survival rates are not promising (78.1% and 70.9%, respectively). All restorations were luted with self-adhesive resin cement or resin-modified glass ionomer cement. Only three clinical trials[16,17] used LD CAD/CAM blocks (IPS e.max CAD) and followed the same surface pretreatment protocol consisting of HF etching and silanization before the application of the resin cement. Reich and Schierz[16] reported a clinically satisfactory success of 96.3% after four years. The crowns were luted using a self-adhesive resin cement (Multilink Sprint, Ivoclar Vivadent). After two years, only one crown showed decementation, but it could be pretreated and recemented again. The authors attributed this failure to the short abutment of <3 mm, but unfortunately it was not possible to examine whether the tooth-resin cement or the resin cement-ceramic interface was weaker. In the clinical trial conducted by Fasbinder and others,[17] 62 chairside posterior crowns were cemented using either a bis-GMA–based, automixed, dual-cure resin cement (Multilink Automix) used with a self-etch adhesive or an experimental self-adhesive resin cement developed by the same manufacturer. Only two (5.1%) of the 39 crowns cemented with the self-adhesive resin cement debonded at two years. The authors reported that the cement was retained on the crown but not on the tooth surface.

The surface treatment, the bonding agent, and the resin cement mediate the link between LD and tooth structure, playing an important role in this



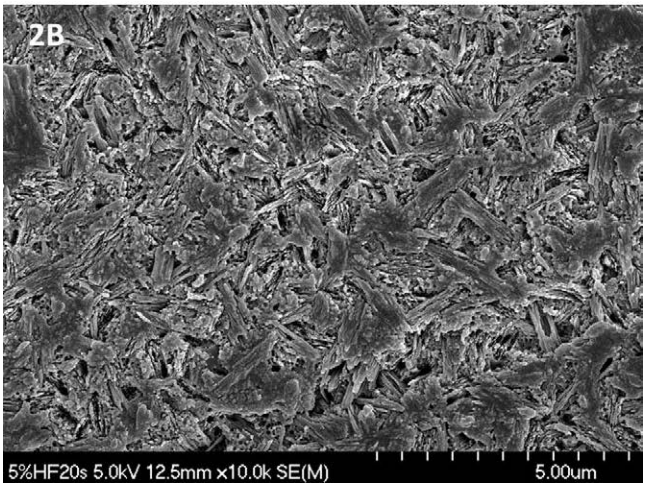
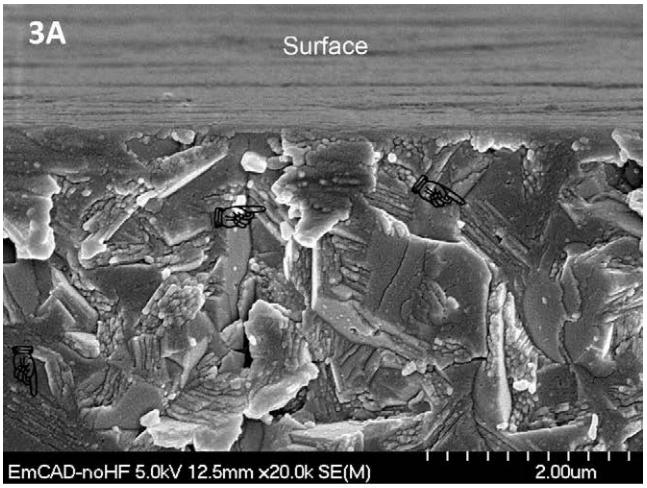


Figure 2. *(A) Micrograph of a polished nontreated LD surface. Original magnification = 10,000×. (B) Polished LD surface after etching with 4.8% HF for 20 sec. Original magnification = 10,000×.*



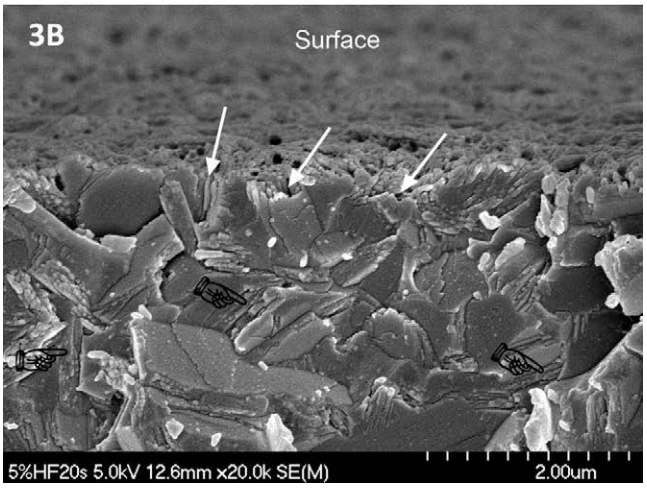


Figure 3. *(A) Cross-sectional view of a polished nontreated LD surface. Original magnification = 20,000×. (B) Cross-sectional view of a polished LD surface after etching with 4.8% HF for 20 seconds. Pointers = LD crystals; Arrows = microretentive grooves from HF dissolution of the LD surface. Original magnification = 20,000×.*

aspect. According to the above-mentioned clinical evidence, HF etching and silanization seem to improve adhesion; however, variations in chemical composition, wetting ability, viscosity, and mechanical properties of each resin cement might also be responsible for variations in the bonding strength. Despite the large number of clinical steps and, consequently, higher probability of operator mistakes, dual-cure resin cements are still considered the "gold standard" for adhesive luting as a result of their increased bond strengths, as reported in various studies.[18,19] The results obtained in our project corroborate this assertion and indicate that adhesion is improved when the complete cementation protocol (HF-Monobond Plus+ExciTE F DSC-Variolink II) is followed. Even though no significant difference was observed when neither silane (HF–no silane–Variolink II) nor adhesive (HF–Monobond Plus–Variolink II) were applied on the etched surfaces, the respective mean μSBS values were not significantly different from those obtained in the nonetched but silanized groups, regardless of adhesive application (NT–Monobond Plus–Variolink II and NT–Monobond Plus+ExciTE DSC–Variolink II). Even at the nonetched LD surfaces, the functional monomers (3-trimethoxysilylpropyl, 10-methacryloyloxi-decyl-dihydrogen-phosphate) present in the silane solution (Monobond Plus) were able to achieve an adhesive strength above 28.0 MPa. In particular, 10-MDP is known for its favorable chemical bonding capabilities to diverse substrates, and it is considered a gold standard monomer for ceramic adhesion.[20] After silanization,

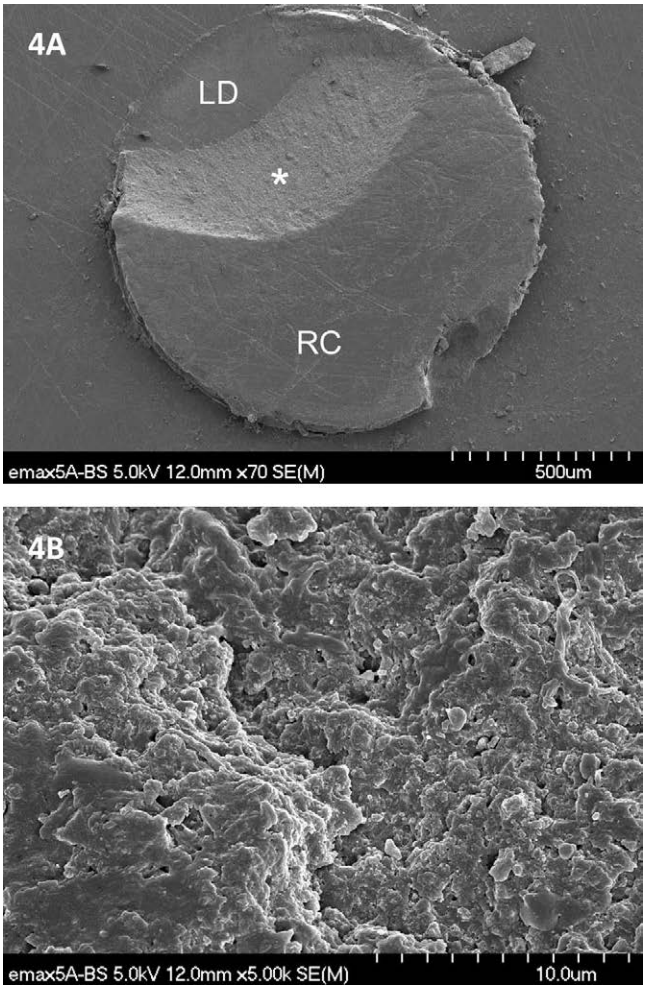


Figure 4. *(A) Mixed failure of group NT+ Monobond Plus + Multilink Automix. LD = Lithium disilicate surface; RC = resin cement; asterisk = area of the RC observed in Figure 4B. Original magnification = 70×. (B) Higher magnification of fracture surface of RC (*) shown in Figure 4A. Original magnification = 5000×.*

the previously hydrophilic surface turns hydrophobic and the luting material is able to optimally wet the LD surface.[21-23] The groups that received HF etching followed by Monobond Plus and Monobond Plus+ExciTE F DSC resulted in 100% mixed failures, which indicates an improved cement interlocking and possible chemical bonding, compared to the other groups that showed close to 50% adhesive failures. A rough and microretentive pattern is created after HF etching, which expands the available surface for bonding (Figures 2B and 3B). These findings are in agreement with those of other studies that found an increase in bond strength after HF etching, even though the manufacturer's recommendations were not strictly followed, specifically as they regard longer etching times and higher HF concentrations.[24-26] However, the 20-second acid-etching time and 4.8% HF concentration (as recommended by the LD manufacturer) were used in the present study because an increase of LD acid-etching time reduces the ceramic flexural strength and modifies the surface roughness.[27]

When a self-adhesive resin cement was used with LD our results suggest that HF and MBP (HF–Monobond Plus–RelyX Unicem 2) should be applied on the LD intaglio surface, as demonstrated by the significant increase in mean μSBS. Beyond that, the extra step consisting of the application of the adhesive system (HF–Monobond Plus+ExciTE F DSC–RelyX Unicem 2) tended to decrease mean μSBS, even though the difference was not statistically significant. Therefore, RelyX Unicem 2 should not be applied if LD is not etched or silanized (NT–no silane–RelyX Unicem 2). However, it must also be taken into account that the chemistry of self-adhesive cements includes an acid-base reaction that is controlled through the presence of water.[10] In fact, a slightly aqueous environment is necessary to improve the ionization of the functional acidic monomers and their bonding capability to dentin.[28,29] During a clinical cementation of a crown, the cement contacts both dentin and ceramic simultaneously just after mixing the catalyst and base pastes. Therefore, it might be possible that during the setting reaction the adhesion to the ceramic surface is influenced by humidity. Further studies should address whether the bond between this resin cement and LD could be improved in the presence of moisture.

The bond between bis-GMA–based, auto-mixed, dual-cure resin cement and LD was clearly improved by silanization (HF–Monobond Plus–Multilink Automix). As with the phosphate monomer–based, self-adhesive, dual-cure resin cement (RelyX Unicem 2), the application of the adhesive system (HF–Monobond Plus+ExciTE F DSC–Multilink Automix) tended to decrease mean μSBS, albeit not significantly. Thus, silanization is mandatory when Multilink Automix is selected for cementation.

One of the limitations of this laboratory study is that the use of resin cement alone does not replicate what may occur clinically, where either tooth structure or build-up core material would be bonded to the intaglio surface. Hence, the stress patterns may be different when the resin cement is applied using a narrow transparent matrix.

CONCLUSIONS

LD benefits from pretreatment of the intaglio surface with HF and silanization, regardless of the resin cement used. Luting LD with a self-adhesive cement without previous HF etching is not recommended.

Acknowledgement

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Conflict of Interest

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

Human Subjects Statement

This study was conducted at the University of Minnesota and the Federal University of Santa Catarina.

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Increased Durability of Resin-Dentin Bonds Following Cross-Linking Treatment

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Clinical Relevance

Topical treatments of etched dentin with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) increase resin-dentin bond stability and may provide better quality and durability to adhesive restorations. EDC used for short periods of time is able to prevent the degradation of the hybrid layer over time and produce long-lasting resin-dentin bonds.

SUMMARY

Objectives: This study evaluated the long-term effect of carbodiimide treatments of acid-etched dentin on resin-dentin bond strength of a simplified etch-and-rinse adhesive system.

Methods: Forty-eight sound third molars were divided into three groups (n=16) according to the dentin treatment: G1: deionized water; G2: 0.5 mol/L 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) applied for 30 seconds; and G3: 0.5 mol/L EDC applied for 60 seconds. Flat dentin surfaces were produced, etched with 37% phosphoric acid for 15 seconds, and then treated with deionized water for 60 seconds or with 0.5 mol/L EDC for 30 or 60 seconds prior to the application of Single Bond 2. Crowns were restored with resin composite, and beam specimens were prepared for microtensile testing. The beams from each group were tested 24 hours or 6 or 12 months after the

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adhesive procedures. One slab from each tooth was prepared and analyzed for nanoleakage. Bond strength (MPa) data were submitted to analysis of variance and Tukey test (α=0.05).

Results: The treatment of dentin with 0.5 mol/L EDC for 30 seconds (24.1±6.2 MPa) and 60 seconds (25.5±5.1 MPa) did not negatively affect the immediate bond strength of Single Bond 2 when compared to the control group (24.6±7.3 MPa). Additionally, EDC prevented resin-dentin bond degradation after 12 months in artificial saliva for both periods of treatment. An increased accumulation of silver ions was seen for the control group over time, while a much lower amount of silver grains was observed for the EDC-treated groups.

Conclusions: 0.5 mol/L EDC was able to prevent resin-dentin bond degradation after 12 months, especially when applied for 60 seconds.

INTRODUCTION

Cross-linking agents have been reported to increase the stiffness of collagen, making it more resistant to degradation.[1-3] These reagents link one peptide chain to another by covalent or ionic bonds.[1,4,5] Endogenous cross-links are naturally present in collagen structure, and its mechanical properties depend on a highly regulated mechanism of intra- and intermolecular cross-linking.[6] Increasing the number of cross-links in dentin collagen by applying exogenous cross-linking solutions prior to adhesive bonding or incorporating these agents into adhesive systems seems to enhance dentin-resin bond durability.[1,3,7,8]

Degradation of resin-dentin bonds is a complex process involving the deterioration of inorganic and organic portions of the hybrid layer.[9,10] Dentin matrix contains proteases, such as matrix metalloproteinases (MMPs),[11-15] that are secreted as inactive proenzyme forms during dental development and are released and activated after acid etching during adhesive bonding.[16] The exposed MMPs on the collagen fibrils at the base of the hybrid layer slowly destroy the collagen fibrils to which they are bound, causing the loss of the anchoring function of hybrid layers and the loss of bond strength of resin composites.[17,18]

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) is a cross-linker that activates free carboxyl groups of glutamic and aspartic acids present in protein molecules[19,20] to form new peptide bonds. It is able to react with collagen[1] and to inactivate matrix-bound MMPs, even when applied on demineralized dentin for periods of time as short as 60 seconds.[21] Therefore, EDC may provide long-lasting adhesive bonds by inactivating MMPs[16,21] and by increasing collagen stiffness.[1]

The purpose of this study was to evaluate the long-term effect of EDC applied for short periods of time on dentin bond strength stability over a period of 12 months. The test null hypothesis was that application of 0.5 mol/L EDC has no significant effect on immediate or long-term dentin bond strength.

METHODS AND MATERIALS

Forty-eight sound human third molars were obtained under a protocol approved by the Ethics Committee of the Araraquara School of Dentistry (protocol #77/11). The occlusal enamel was completely removed from each tooth to obtain flat dentin surfaces using an ISOMET saw (Buehler Ltd, Lake Bluff, IL, USA) under water cooling. Then the smear layer thickness was standardized by wearing the dentin surface with 320-grit silicon carbide sandpaper, and the teeth were randomly divided into three groups, according to the dentin treatment (n=16): G1: deionized water applied for 60 seconds (control); G2: 0.5 mol/L EDC applied for 30 seconds; and G3: 0.5 mol/L EDC applied for 60 seconds.

Bonding Procedures

The dentin was etched with 35% phosphoric acid (Scotchbond etchant, 3M ESPE, St Paul, MN, USA) for 15 seconds and then rinsed with deionized water for the same time. After blot drying, 20 μL of deionized water or 0.5 mol/L of EDC prepared in Sorensen's buffer (pH 6) were applied on demineralized dentin for 30 or 60 seconds and then rinsed for 15 seconds. The excess of water was removed from the surface with absorbent paper prior to bonding. The adhesive system Single Bond 2 (Z350, 3M ESPE) was applied according to the manufacturer's instructions, except for the dentin treatments, and photoactivated for 10 seconds (Radii Plus, SDI Ltd, Bayswater, Victoria, Australia; 1000±10 mW/cm^2). A 3-mm-high resin composite block (Z350, 3M ESPE) was built up incrementally, and each increment was light cured for 20 seconds using the LED curing light. The restored teeth were then stored in deionized water and kept in an incubator at 37°C for 24 hours.

Table 1. *Bond Strength (MPa) of Single Bond 2 to Dentin After Treatment with 0.5 mol/L EDC for 30 or 60 Seconds and Storage in Artificial Saliva for Up to 12 Months.*[a]

Dentin Treatment	Storage Period		
	24 h	6 Mo	12 Mo
Water (control)	24.6 ± 7.3 AB	21.1 ± 3.8 CD	18.5 ± 6.5 D
EDC 30 s	25.1 ± 6.2 AB	22.3 ± 4.8 BC	21.2 ± 4.7 BD
EDC 60 s	26.7 ± 5.1 A	25.9 ± 4.6 A	27.9 ± 7.2 A

[a] *Numbers are mean ± standard deviation, n = 16. Groups identified by the same letter are statistically similar (Tukey test,* p>*0.05).*

Microtensile Bond Strength Testing

After 24 hours in deionized water, dentin beams with a cross-sectional area of 0.81 mm^2 were obtained in a high-precision cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) using a diamond saw (Isomet, Buehler Ltd) under water cooling. One-third of the beams from each tooth were tested 24 hours after the bonding procedures. The remaining specimens were stored in 3 mL of artificial saliva at 37°C for 6 or 12 months before the microtensile test. The artificial saliva pH was monitored periodically to ensure that no significant changes occurred. The cross-sectional area of each beam was individually measured (Model 500-144B, Mytutoyo South America Ltda, SP, Brazil) and the beam fixed to a testing device with cyanoacrylate glue (Super Bond Gel, Henkel Loctile, São Paulo, SP, Brazil) and subjected to the microtensile strength test in a mechanical testing machine (DL-Digital Line, EMIC, Parana, Brazil) equipped with a 100-N load cell running at a crosshead speed of 1.0 mm/min. Immediately after testing, the debonded halves of each beam were dried and stored in closed receptacles at room temperature until analysis of the fracture pattern using a stereomicroscope (Carl Zeiss, Oberkochen, Germany) at approximately 50× magnification. Failures were classified as cohesive in resin or dentin, adhesive or mixed.

Nanoleakage Analysis

In each group, four 0.9-mm-thick slabs were randomly selected and immersed in 50 wt% ammoniacal $AgNO_3$ solution (pH 9.5) in darkness for 24 hours according to the protocol described by Tay and others.[22] After immersion in the tracer solution, followed by rinsing in deionized water for 5 minutes, the slabs were immersed in photodeveloping solution for 8 hours under a fluorescent light to reduce silver ions into metallic silver grain. The silver-stained specimens were polished with silicon carbide sandpaper with different grits (400, 600, 1200, 2400, and 4000). Then they were cleaned, mounted on aluminum stubs, and placed in a desiccator for 24 hours. Digital images were obtained using scanning electron microscopy (SEM; XL-30 FEG, Philips, Eindhoven, The Netherlands) with a back-scattered electron detector at 10 kV at 2500× magnification.

Statistical Analysis

Bond strength recorded for specimens obtained from the same tooth were averaged in such a way that the tooth was used as the statistical unit of the study. Premature failures (failures that occurred before testing) were included in the computation of the mean as zero (0 MPa). Two-way analysis of variance (ANOVA) and Tukey tests were applied to analyze the effect of dentin treatment and storage period on microtensile bond strength (MPa). The significance level was 5% for all analyses. Failure mode and nanoleakage data were analyzed descriptively.

RESULTS

Microtensile Bond Strength

The ANOVA test showed a significant effect of dentin treatment (p=0.001) and interaction between dentin treatment vs storage period (p=0.041). No significant effect was detected for storage period (p=0.653). Bond strength data (MPa) are shown in Table 1 as mean and standard deviation. No statistically significant difference was observed among the immediate bond strength of Single Bond 2 after the treatment of dentin with deionized water (control) or 0.5 mol/L EDC 30 or 60 seconds. After 6 months in artificial saliva, significant bond strength decrease was observed only for the control. Bonds made to EDC-treated dentin showed bond strength values that were significantly higher than 6-month controls when EDC was applied for 60 seconds. The beams treated with deionized water (control) and stored for 12 months showed a significant reduction in bond strength when compared to that observed after 24 hours. The dentin treated with EDC for 30 or 60 seconds presented no statistically significant decrease in bond strength of Single Bond 2 after 12 months of artificial saliva storage. However, at the 12-month storage period, higher mean bond strength was seen for EDC-treated dentin at 60 seconds.

Failure Modes and Nanoleakage Analysis

The distribution of failure modes is given in Table 2 as absolute values and percentage of occurrence within the group. The largest percentages of failures

Table 2. *Distribution of Failure Types*

Dentin Treatment	Storage Period		
	24 h	6 Mo	12 Mo
Water (control)	A = 64 (88.9); M = 1 (1.4); CR = 1 (1.4); CD = 1 (1.4); PF = 5 (6.9)	A = 47 (83.9); M = 3 (5.4); PF = 6 (10.7)	A = 67 (82.7); M = 2 (2.5); CR = 1 (1.2); CD = 1 (1.2); PF = 10 (12.3)
EDC 30 s	A = 78 (95.1); M = 1 (1.2); CD = 2 (2.4); PF = 1 (1.2)	A = 57 (75.0); M = 15 (19.7); PF = 4 (5.3)	A = 76 (82.6); M = 3 (3.3); CR = 3 (3.3); CD = 2 (2.2); PF = 8 (8.7)
EDC 60 s	A = 68 (95.8); CD = 2 (2.8); PF = 1 (1.4)	A = 59 (83.1); M = 8 (11.3); PF = 4 (5.6)	A = 63 (78.8); M = 4 (5.0); CR = 1 (1.3); CD = 5 (6.3); PF = 7 (8.8)

Abbreviations: A, adhesive; M, mixed; CR, cohesive in resin; CD, cohesive in dentin; PF, premature failure. Values represent the absolute frequency (percentage of total specimens in the group).

involved the interface, adhesive, and mixed fractures. Premature failures were observed in all groups and increased as a function of storage period. EDC seems to not influence resin-dentin bond failure mode.

Representative SEM photomicrographs of silver nanoleakage in adhesive bonds created by Single Bond 2 on dentin surfaces treated with water (control group) or 0.5 mol/L EDC for 30 or 60 seconds and stored for 24 hours 6 or 12 months at 37°C are presented in Figure 1. SEM images revealed the presence of silver deposits in all groups and storage periods. The specimens analyzed 24 hours after the

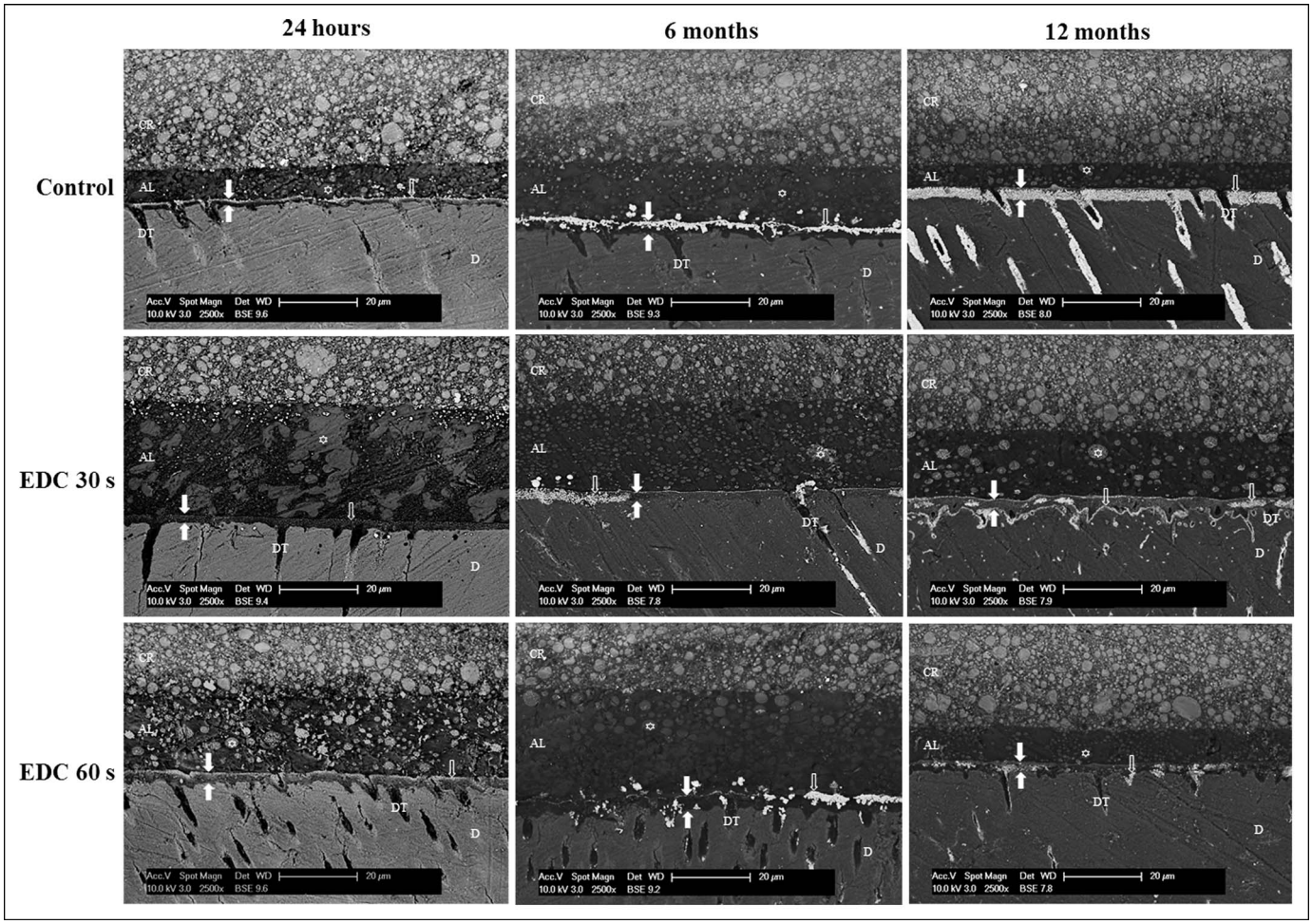

Figure 1. *SEM photomicrographs of adhesive bonds created by Single Bond 2 on dentin surfaces pretreated with water (control group) or 0.5 mol/L EDC for 30 or 60 seconds and stored in artificial saliva for 24hours or 6 or 12 months. The zone between white filled arrows represents the hybrid layer (HL).* ⇑ *shows silver deposits in the HL, and* ☆ *shows polyalkenoic acid copolymer globules. AL, adhesive layer; D, mineralized dentin; CR, composite resin; DT, dentin tubule. Magnification 2500×.*

bonding procedures showed small silver accumulation. In the control group, a gradual increase in nanoleakage was observed over time. However, the groups treated with EDC for 30 or 60 seconds showed much lower amounts of silver nanoleakage, even after 12 months in the artificial saliva. All the SEM images exhibited the presence of polyalkenoic acid copolymer globules in the adhesive layer of Single Bond 2, indicating the occurrence of phase separation.

DISCUSSION

Collagen fibrils in mineralized dentin are protected from hydrolytic and enzymatic degradation by hydroxyapatite crystals. However, after acid etching, collagen fibrils become uncovered, and their bound proteases (MMPs and cathepsins) are activated[23] to cleave unprotected collagen, resulting in a decrease in long-term bond strength,[24] as was observed in the control group of this study.

In order to reduce the activity of these proteases and preserve the integrity and durability of the adhesive interfaces, chlorhexidine (CHX) has been used as a nonspecific MMP[18,24-28] and cathepsin[29] inhibitor. However, chlorhexidine is highly water soluble and may be leached from the hybrid layer, compromising its antiprotease efficacy.[24] The use of cross-linking agents instead of CHX has the advantage of inactivating endogenous MMPs[21,30] and simultaneously increasing collagen mechanical properties[1] by creating covalent cross-links that are stable over time.

The biomodification of collagen by extrinsic cross-linkers can induce the formation of additional inter- and intramolecular cross-links,[31,32] increasing the ultimate tensile strength and elastic modulus of demineralized dentin.[33,34] Cross-linking agents such as proanthocyanidins, glutaraldehyde, and tannic acid are able to[7,35] improve immediate resin-dentin bond strength after 1 hour of treatment, an observation that was not seen in our results. However, we used much shorter periods of treatment. The present study showed that 0.5 mol/L EDC applied on dentin for short periods of time, such as 30 and 60 seconds, was capable of preventing resin-dentin bond degradation after up to 12 months of aging in artificial saliva. These results require partial rejection of the tested null hypothesis. These findings agree with those of Mazzoni and others,[36] which showed that the application of 0.3 mol/L EDC on demineralized dentin produces long-term inactivation of MMPs, contributing to bond strength preservation over time.

To degrade the organic matrix of dentin, MMPs must link their narrow binding sites[37] to the substrate and unwind collagen molecules,[38-40] culminating in collagen peptide cleavage. However, the treatment of demineralized dentin with cross-linking agents makes collagen more difficult to unwind, preventing the degradation by MMPs. EDC activates the free carboxylic groups of glutamic and aspartic acids present on collagen[1] and MMP structures.[21] It increases collagen stiffness and inactivates MMP activation sites.[21] Additionally, the cross-links created by EDC may reduce the mobility of these enzymes.

Single Bond 2 is a two-step etch-and-rinse adhesive system that contains hydrophilic resin monomers to enhance the adhesive wetting properties and avoid phase changes observed when hydrophobic monomers are added to water.[41] Therefore, these adhesives have a high water affinity[42,43] that favors their degradation.[42] Over time, it is thought that infiltrated resins are extracted from dentin matrix[44,45] and that uninfiltrated collagen fibrils are hydrolyzed and replaced by water.[46] The silver nanoleakage protocol fills water spaces with silver nitrate that is later photoreduced to silver grains that can be analyzed by SEM.[22] After 6 and 12 months in artificial saliva, the control group showed higher silver accumulation compared to the EDC-treated groups. These results indicate that there was a greater degradation of the hybrid layer in the control group. Conversely, the cross-linking agent applied for 30 and 60 seconds on etched dentin was able to prevent the increase in nanoleakage.

Bond strength data and nanoleakage images showed that the treatment of demineralized dentin with EDC could be a simple, practical, and clinically applicable method to reduce collagen degradation in the hybrid layer, being an efficient alternative to make resin-dentin bonds more durable. How much of the increase in durability is due to cross-linking of collagen vs collagen-bound MMPs and cathepsins is unclear. Further studies are needed to better understand the effects of EDC application *in vitro* and to demonstrate its efficacy *in vivo*.

CONCLUSION

The treatment of acid-etched dentin with 0.5 mol/L EDC prior to bonding procedures was able to prevent resin-dentin bond degradation after up to 12 months especially when applied for 60 s.

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Conflict of Interest

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

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One-year Adhesive Bond Durability to Coronal and Radicular Dentin Under Intrapulpal Pressure Simulation

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Clinical Relevance

A two-step self-etch adhesive maintains high bonding strength to both coronal and radicular dentin even after long-term storage under intrapulpal pressure simulation.

SUMMARY

Objective: To evaluate the microtensile bond strength (μTBS) of different adhesives to coronal vs radicular dentin after one year of storage in artificial saliva and under intrapulpal pressure (IPP) simulation.

Methods and Materials: Roots of 36 freshly extracted premolars were sectioned 5 mm apical to the cemento-enamel junction and pulp tissue was removed. Buccal enamel and cementum were trimmed to obtain standardized flat dentin surfaces. Specimens were divided into three groups (n=12/group) according to the adhesive strategies utilized: a two-step etch-and-rinse adhesive; a two-step self-etch adhesive; and a single-step self-etch adhesive. Adhesives and resin composite were applied to coronal and radicular dentin while the specimens were subjected to IPP simulation. After curing, specimens were stored in artificial saliva at 37°C in a specially constructed incubator while the IPP was maintained for either 24 hours or one year prior to testing. Bonded specimens were sectioned into sticks with a cross section of 0.8 ± 0.01 mm^2 and subjected to μTBS testing. Data were statistically analyzed using multi-way analysis of variance (ANOVA) with repeated measures; one-way ANOVA tests; and Bonferroni post hoc test ($p<0.05$). Failure modes were determined using a scanning electron microscope at 100× magnification.

Results: ANOVA results revealed a statistically significant effect for the adhesive strategy ($p<0.001$) and storage period ($p<0.001$) as well as for their interaction ($p=0.024$) on the μTBS. However, dentin substrate and its interactions

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revealed no significant effects. For both dentin substrates, the two-step self-etch adhesive revealed statistically significantly higher μTBS values than did the other two adhesives after 24 hours and one year of storage. After one-year storage, a significant decrease in bond strength values of all tested adhesives occurred with both dentin substrates. Modes of failure were mainly adhesive and mixed.

Conclusions: Adhesives were not sensitive to structural differences between coronal and radicular dentin even after one year of storage under IPP simulation. However, all tested adhesive systems strategies were sensitive to storage.

INTRODUCTION

New adhesive systems have been developed in an attempt to reduce the steps and simplify the clinical bonding procedures. However, one of the challenges facing the adhesive systems' manufacturers has been, and still remains, the development of adhesive agents that adhere equally well to different tooth substrates.

Dentin is a biologic composite structure composed of apatite filler crystallites in a collagen matrix with a fluid-filled tubular structure connecting the pulp to the dentino-enamel junction. This heterogeneous and intrinsically wet substrate changes with different dentin depths and varies from location to location.[1] The use of coronal dentin is adequate as a means to obtain information about the bonding efficacy of any material. However, in the clinical situation, bonding is performed to dentin, which is located at various sites. Recent developments in preventive dentistry and periodontology have considerably increased the demand for restoration of root dentin defects such as cervical erosion, abrasion, and root caries.[2,3] In root dentin, there is a significant reduction in the average density of dentinal tubules running in a straight course compared with coronal dentin tubules running in an "s"-shaped course.[4] These variations in density and morphology of dentinal tubules were reported to affect the interaction between earlier versions of dentin adhesives with different dentin sites.[5,6]

Another issue is related to the outward fluid movement through dentinal tubules, which is one of the most critical differences between clinical and laboratory conditions.[7] As a consequence, it is necessary to employ pulpal pressure simulation when adhesives are tested *in vitro*. On reviewing the literature, no research has been conducted to determine the long-term bond durability to coronal and radicular dentin when intrapulpal pressure (IPP) is simulated. The null hypotheses were the following: 1) Bonding to coronal or radicular dentin has no influence on adhesive bond strength. 2) There is no difference in the microtensile bond strength among different adhesive systems of different bonding strategies. 3) Storage under IPP simulation has no effect on bond strength of different adhesives to dentin.

METHODS AND MATERIALS

Specimen Preparation

Thirty-six sound human lower premolar teeth, extracted for orthodontic reasons from young patients (14-17 years), were collected and stored in phosphate buffer solution containing 0.02% sodium azide at 4°C for not more than one month until they were used. The roots were trimmed perpendicular to the long axis of the teeth, leaving 5 mm apical to the cemento-enamel junction (CEJ). The pulp tissue was removed from the pulp chamber using a broach (Mani Inc, Utsunomya Tochgi, Japan), size 35,[8] and then the pulp chamber was irrigated with saline solution to ensure complete cleanliness of the chamber.[9] Each tooth segment was fixed perpendicularly from the cut root surface to the center of a circular Teflon plate (11-mm diameter and 1.5-mm thickness, with a central hole of 1-mm diameter) using a cyanoacrylate adhesive (Rocket Heavy, Dental Ventures of America Inc, Corona, CA, USA). A 19-gauge stainless-steel butterfly needle (Shanchuan Medical Instruments. Co, Ltd, Zibo, China) was verified to penetrate the plate to reach the root canal of the tooth. A line was drawn with an indelible pen demarcating the middle of the proximal surface. Another line was drawn at the CEJ to differentiate between coronal and root dentin surfaces. The tooth segment attached to the Teflon plate was horizontally embedded in a polyester resin (Polyester resin #2121, Hsein, Taiwan) up to the level of the middle of the proximal surfaces that was demarcated, while the lingual surface was facing downward and the buccal surface was facing upward. The needle was left inserted in the root canal during embedding to guarantee a patent pathway to the pulp chamber. Buccal enamel and dentin were then trimmed parallel to the tooth long axis using a slow-speed diamond saw sectioning machine (Buehler Isomet Low Speed Saw, Lake Bluff, IL, USA) under water coolant to obtain a standardized flat dentin surface.

Table 1: *Materials, Compositions, and Application Procedures*

Material (Manufacturer)	Composition	Application procedures
Adper Single Bond 2 • Two-step etch-and-rinse adhesive system • 3M ESPE Dental Products, St Paul, MN, USA Batch#51202	*Etchant:* 35% Phosphoric acid, colloidal silica. *Adhesive:* Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymers of polyacrylic and polyitaconic acids, silica nanofillers.	*Etching:* Apply for 15s, water rinsing for 10s then blot excess water with minisponge (visibly moist surface). *Adhesive:* Apply with gentle agitation for 15s, gently air-thin for 5s and light cure for 10s.
Clearfil SE Bond • Two-component two-step self-etch adhesive system • Kuraray Medical Inc. Sakazu, Kurashiki, Okayama, Japan Primer: Batch #00999A Adhesive: Batch #01486A	*Primer:* MDP, HEMA, hydrophilic dimethacrylate, D,L-camphorquinone, N,N-diethanol-p-toluidine and water. *Bond:* MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine and silanated colloidal silica	*Primer*: Apply onto the visibly moist prepared tooth surface, leave undisturbed for 20s and then dry with oil-free mild air flow for 5s. *Bond*: One coat application and a gentle oil-free air stream for 5s then light cure for 10s.
Adper Easy One • (One-component single-step self-etch adhesive system) • (3M ESPE Dental products, Seefeld, Germany) Batch #D-82229	HEMA, Bis-GMA, methacrylated phosphoric esters, 1.6 hexanediol dimethacrylate, methacrylate functionalized polyalkenoic acid (Vitrebond copolymer), finely dispersed bonded silica filler with 7nm primary particle size, ethanol, water, initiators based on camphorquinone, stabilizers.	Apply with the disposable mini-sponge brush tip for 20s to the whole dentin surface, then air thin with oil-free mild air flow for 5s until the film no longer moves, and cure for 10s.

Bis-GMA=Bis-phenol-A glycidyl methacrylate, HEMA=2-hydroxyethyl methacrylate, MDP=10-Methacryloyloxydecyl dihydrogen phosphate.

The dentin surfaces were hand finished with wet 600-grit silicon carbide (SiC) abrasive paper for 20 seconds to obtain a clinically relevant uniform smear layer. The drawn line identifying the CEJ was regained to differentiate between coronal and root surface dentin. The teeth segments (n=36) were connected to the IPP assembly during bonding and storage following the same procedures described by Mobarak.[10]

Restorative Procedures

Prepared teeth segments with flattened dentin surfaces (coronal and radicular) were divided into three subgroups (n=12) according to the adhesive system strategies evaluated: a two-step etch-and-rinse adhesive system (Adper Single Bond 2, SB, 3M ESPE Dental Products, St Paul, MN, USA); a two-component two-step self-etch adhesive system (Clearfil SE Bond, SE, Kuraray Medical Inc, Okayama, Japan); and a one-component single-step self-etch adhesive (Adper Easy One, AE, 3M ESPE Dental Products, Seefeld, Germany). Each adhesive system was applied to moist dentin surfaces according to its manufacturers' instructions, as described in Table 1. Resin composite (Valux Plus, 3M ESPE Dental Products) of shade A3.5 was applied in two increments of 1.5 mm each, building up two blocks of resin composite (approximately 3 mm in height and 3 mm in length), to the prepared coronal and radicular dentin, where a matrix band was placed to separate surfaces. Each composite increment was polymerized for 40 seconds using a Bluephase C5 light curing unit (Ivoclar Vivadent, Schaan, Liechtenstein) with an intensity of $\geq$500 mW/cm^2. Light intensity was checked using an LED radiometer (Kerr Dental Specialties, Orange, CA, USA). The specimens were then immersed in artificial saliva either for 24 hours (n=6) or one year at 37°C in a specially constructed large incubator to accommodate the IPP assembly. The artificial saliva was prepared according to Pashley and others[11] and was changed weekly.[8]

Microtensile Bond Strength Testing

Before specimen sectioning, the coronal composite build-up was color-coded to guarantee the differentiation of the sticks after sectioning. Each bonded tooth was sectioned in the X and Y axes to obtain sticks of 0.8 $\pm$ 0.01 mm^2 for the microtensile bond strength (μTBS) test. From each specimen, sticks of similar length and remaining dentin thickness (four for coronal and four for radicular) were selected; thus, a total of 24 sticks of each experimental variable were tested. Each stick was fixed to the modified ACTA microtensile strength jig[12] with a cyanoacrylate adhesive (Rocket Heavy) and stressed in tension using a universal testing machine (Lloyd Instruments Ltd, Ametek Company, Bognor Regis,

Table 2: *Microtensile Bond Strength (μTBS) Values mean (standard deviation) in MPa of the Tested Adhesive Systems*[a]

Storage Periods	Coronal dentin			*P*-Value
	Adper Single Bond 2	Clearfil SE Bond	Adper Easy One	
24 hours	30.7 (5.2) A [Ptf/tnt=1/24]	39.9 (9.1) B [Ptf/tnt=0/24]	25.7 (3.4) A [Ptf/tnt=2/24]	<0.01
1 year	13.0 (2.6) A [Ptf/tnt=8/24]	29.4 (3.9) B [Ptf/tnt=2/24]	12.1 (3.3) A [Ptf/tnt=9/24]	<0.001
p-value	<0.0001	0.001	0.037	

[a] *[ptf/tnt=pretest failure/total number of tested sticks]. Within rows, for each dentin substrate, means with different capital letters are statistically significantly different (p>0.05, Bonferroni test).*

West Sussex, UK) at a cross-head speed of 0.5 mm/min until failure. The tensile force at failure was recorded and converted to tensile stress in MPa units using computer software (Nexygen-MT, Lloyd Instruments). Sticks that failed before testing were counted as zero MPa.[8]

The mean and standard deviation (SD) of each group were calculated. Comparison between groups was performed using the multi-way analysis of variance (ANOVA) with repeated measures where μTBS was the dependent variable and the dentin site, adhesive strategies, and storage periods were the independent variables. The interactions between each of the two independent variables as well as the interaction among the three variables were also tested. A Bonferroni post hoc multiple-comparison test was used when indicated. A *t*-test was used to compare the bond strength values of 24-hour and one-year μTBS mean values for each adhesive system with each dentin site. A *p*-value of <0.05 was considered statistically significant. Data were analyzed using SPSS for Windows (Statistical Package for Social Sciences, release 15 for MS Windows, 2006, SPSS Inc, Chicago, IL, USA).

Failure Mode Analysis

The fractured dentin side of all tested sticks was inspected under scanning electron microscopy (SEM) (Scanning Electron Microscope 515; Philips, Einhoven, The Netherlands) to determine the mode of failure. The failure mode was allocated to either type 1: adhesive failure at dentin side; type 2: cohesive failure in the adhesive layer; type 3: mixed failure (adhesive at dentin side/cohesive in the adhesive layer); or type 4: mixed failure (adhesive at dentin side/cohesive in the adhesive layer/cohesive in resin composite). The frequency of each mode of failure was expressed as a percentage value.[13]

SEM Observation of the Bonded Coronal and Radicular Dentin Interfaces

An additional two sticks from each tested category were randomly selected for evaluation of the interfacial morphology using SEM (515; Philips). Sticks were polished using SiC paper of increasing grit size (1000, 1200, 2500, and 4000), rinsed with water for 30 seconds, etched with 10% phosphoric acid for 10 seconds; and deproteinized in 5% sodium hypochlorite for five minutes. After rinsing with distilled water, sticks were left to air-dry in a dessicator; they were then mounted on aluminum stubs and sputter-coated with gold to be examined using SEM at different magnifications.

RESULTS

Multi-way ANOVA with repeated measures revealed a significant effect for the adhesive strategies ($p<0.001$) and storage periods ($p<0.001$) as well as for their interactions ($p=0.024$) on the μTBS. However, dentin site and its interactions revealed no significant effects. The descriptive statistics, means, and SDs for the μTBS (MPa) of all tested categories are presented in Table 2. The one-way ANOVA test also indicated that there was a significant difference among the adhesive systems with both dentin substrates when tested after 24 hours and after one year of storage (Table 2). The two-step self-etch adhesive (SE) revealed the highest mean bond strength compared with the two-step etch-and-rinse adhesive (SB) and the single-step self-etch adhesive (AE). The Bonferroni post hoc test revealed that SB and AE were significantly lower than SE at 24 hours as well as at one year. With regard to the effect of storage period, the *t*-test revealed a significant decrease in bond strength values of all tested adhesive systems to coronal and radicular dentin after one year of storage (Table 2).

Regarding the failure modes, Figure 1 shows the percentages of the recorded failure modes. For both dentin sites, after 24 hours of storage, fractured specimens for all adhesive system showed mainly a type 1 mode of failure. After one-year storage, for both dentin sites, SB fractured specimens showed predominantly a type 3 mode of failure; SE fractured specimens showed mainly types 1 and 3 modes of failure, while for the AE fractured specimens, failure

Table 2: *Microtensile Bond Strength (μTBS) Values (standard deviation) in MPa of the Tested Adhesive Systems[a] (ext.)*

Storage Periods	Radicular dentin			*P*-Value
	Adper Single Bond 2	Clearfil SE Bond	Adper Easy One	
24 hours	28.1 (4.5) A [Ptf/tnt=0/24]	43.3 (8.5) B [Ptf/tnt=0/24]	22.3 (3.0) A [Ptf/tnt=5/24]	<0.001
1 year	10.1 (2.3) A [Ptf/tnt=7/24]	32.7 (3.9) B [Ptf/tnt=2/24]	15.6 (3.7) A [Ptf/tnt=7/24]	<0.001
p-value	0.028	0.01	<0.01	

mode types 1 and 4 were mainly recorded. Representative SEM micrographs for the predominant failure modes recorded with tested adhesive systems bonded to either coronal or radicular dentin are presented in Figure 2.

Figure 3 shows the SEM images of the bonded coronal and radicular interfaces. Characteristic pictures for SB (etch-and-rinse) specimens were captured in which resin tags with conical swellings were detected. For the self-etch adhesives (SE and AE) specimens, SEM images showed uniform homogeneous hybrid layer and adhesive layer thickness.

DISCUSSION

Results of the present study indicate that the first null hypothesis should not be rejected, as there was no difference between coronal and radicular dentin bond strength values at 24 hours or at one year of storage. Earlier studies,[14,15] indicated opposite results where lower bond strength values of root dentin were recorded compared to those of coronal dentin surfaces. They attributed this difference in bonding to a decrease in number and diameter of dentinal tubules as well as permeability in root dentin relative to those of coronal dentin which might reduce the hydrophilic resin infiltration capacity of adhesives.

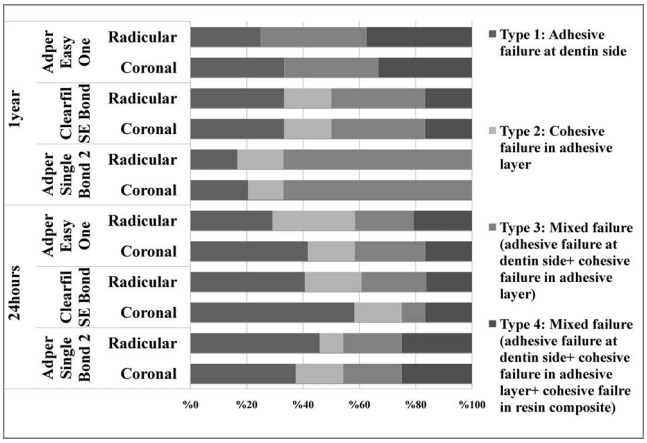

Figure 1. *Percentage failure modes of the tested specimens.*

On the contrary to previous findings and in agreement with part of our findings, Pazinatto and others[16] did not find any influence of tubule orientation or dentin site on adhesive bond strength to dentin. It is important to emphasize that all earlier studies did not test coronal and radicular dentin bond strength under simulated IPP and were done over short-term periods. In the present study, bonding and storage of the specimens were done under IPP simulation. Although IPP simulation in other studies has influenced dentin bond strength,[17,18] the difference in dentin site and dentinal tubule orientation did not show a significant effect in the present study even after one year of storage under IPP simulation.

Regardless of dentin sites, dentin bond strengths of tested adhesive systems were significantly different leading to the rejection of the second null hypothesis. Long-term storage under IPP simulation significantly decreased bond strengths of all adhesives which suggested the rejection of the third null hypothesis.

Many authors[16,19,20] confirmed that dentin bond strength is adhesive dependent. After 24 hours and one year, SE (the two-step self-etch adhesive system)

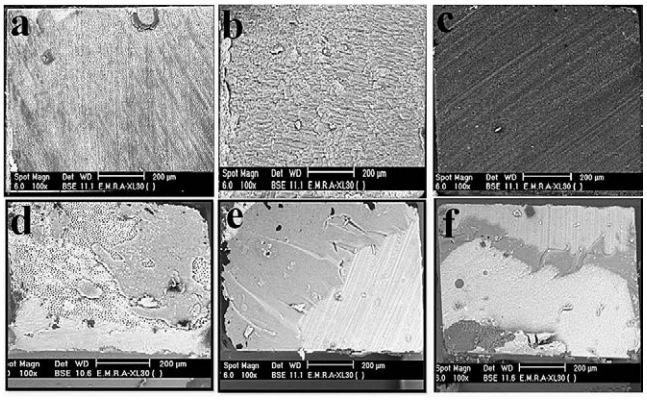

Figure 2. *Representative scanning electron photomicrographs showing the predominant failure modes of Adper Single Bond 2, Clearfil SE Bond and Adper Easy One/coronal dentin fractured specimens (a, b and c, respectively). While (d, e and f) are the representative SEMs of the predominant failure modes of Adper Single Bond 2, Clearfil SE Bond and Adper Easy One/radicular dentin fractured specimens, respectively.*

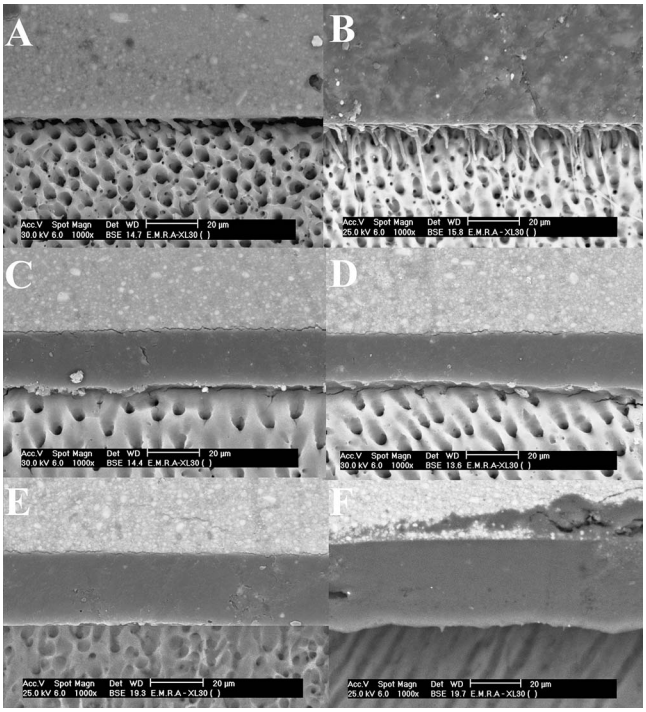

Figure 3. *Representative scanning electron photomicrographs showing the bonded interfaces of Adper Single Bond 2, Clearfil SE Bond and Adper Easy One/coronal dentin specimens (A, C and E, respectively) and Adper Single Bond 2, Clearfil SE Bond and Adper Easy One/radicular dentin specimens (B, D and F), respectively.*

showed the highest significant bond strength values in comparison to the other two adhesive systems, which in turn were comparable with no statistically significant difference.

The lower bond strength of the two-step etch-and-rinse adhesive (SB) compared to the two-step self-etch adhesive (SE) was in accordance with others[2,9,21,22] despite that bonding was done in their studies to coronal dentin at different locations. Bonding to parallel-cut dentin implies that the numbers of resin tags, which have been postulated to contribute to 25% of the recorded bond strength values, are reduced.[23] Also, the smear layer removal with etch-and-rinse adhesive systems increases dentin hydraulic conductance allowing the outward flow of the fluid within the dentinal tubule to the surface of the dentin. This renders the etch-and-rinse adhesive systems to be very sensitive to IPP simulation. The debonded specimens of SB, showed predominately adhesive failure at the dentin side for both coronal and root dentin groups which support that IPP simulation caused excessive moisture on the adherent substrate.

Conversely, the self-etch adhesive system is expected to be less influenced by IPP simulation. The etching effects of self-etch adhesives depend on the concentration and pH of their acidic monomers. The mild etching effect imposed by the self-etching primer (pH≈2.0) of SE results in residual mineral crystals within the hybrid layer and maintains smear plugs blocking the tubule orifices. This fact, combined with the use of a separate, relatively hydrophobic, solvent-free adhesive layer placed over the hydrophilic primer, significantly reduces the rate of fluid flow through the interface even in the presence of IPP.[17] This was confirmed by Hashimoto and others[24] who reported that although the smear layer and smear plugs do not provide an impermeable or hermetic seal of the dentinal tubules, they account for up to 86% of the total resistance to fluid movement across dentin.[25] Another reason for the highest bond strength values of the two-step self-etch SE adhesive system could be due to the presence of an unsaturated methacrylate phosphate ester 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) as an acidic monomer together with HEMA which is believed to improve the wetting of SE adhesive to moist dentin.[17,26] A molecule like 10-MDP has high affinity to chemically bond to the calcium in the hydroxyapatite which could have played a part in recording high bond strength.[27]

To explain the inadequate performance of the single-step self-etch adhesive, compared with the two-step self-etch adhesive (SE), some major differences should be elicited. Single-step self-etch adhesives were found to contain high concentrations of HEMA which induces the formation of a HEMA-rich oxygen-inhibition layer that may enhance the osmotic process of water movement.[28] Moreover, they contain mainly hydrophilic monomers which may cause reduction in polymerization due to their dilution with water flow from the bonded dentin.[29] The intrinsic hydrophilicity renders them more sensitive to water contamination, even though they preserve the smear layer. Moreover, the dense distribution of polar hydrophilic domains within these adhesives increases sites for water binding and transport.[30]

Mode of failure of the single-step self-etch adhesive specimens supported the microtensile bond strength results as they revealed a higher percentage of cohesive failure in the adhesive layer with both coronal and radicular dentin surfaces. As previously observed by Belli and others,[30] HEMA-containing single-step self-etch adhesives have shown clear evidences of water uptake and droplet accumulation at the adhesive/composite interface.

After one year of storage in saliva immersion at 37°C and under IPP simulation, interfacial bond strengths of all adhesive systems were significantly affected. Abdalla and others[17] and El-Deeb and others[8] reported a significant decrease in the two-step self-etch adhesive (SE) bond strength after six-month storage under IPP simulation. They referred this reduction to slow water sorption of the adhesive which could affect its mechanical properties and thus its bond strength. On the contrary, another study[30] reported no statistically significant difference between adhesive dentin bond strength values after one-year storage under IPP simulation. The application of simulated IPP in that study was restricted to the storage period while in the present study IPP was applied during the bonding procedure and over the storage period. Although bond strength of SE significantly decreased after storage, it maintained the highest value compared to the other adhesive systems. The formed calcium-phosphate salts along with only a limited surface-decalcification effect was referred to be more stable to hydrolytic degradation.[27]

Present study findings emphasized that current bonding strategies could surpass circumstances of regional variability, dentin site, as well as intrinsic and extrinsic moisture that present in the oral cavity. However, improving adhesive systems to provide successful and durable restorations is still required.

CONCLUSIONS

Bond strengths of tested adhesives were not sensitive to structural differences between coronal and radicular dentin even after one-year storage and under IPP simulation. However, all tested adhesive systems were sensitive to storage.

Human Subjects Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies. The approval code for this study was 112/2011. This study was conducted at the Faculty of Oral and Dental Medicine, Cairo University.

Conflict of Interest

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

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Effect of Desensitizing Agents on the Bond Strength of Mild and Strong Self-etching Adhesives

C Sabatini • Z Wu

Clinical Relevance

When a desensitizing agent is indicated prior to bonding, clinicians should be aware of its potential effect on the bond strength to dentin.

SUMMARY

Background: Desensitizing agents are used, almost as routine practice, in many adhesive restorative procedures. There is still debate as to their effect in dentin bonding, particularly with self-etching adhesives. The present study aimed to evaluate the effect of different desensitizing agents on the bond strength of mild and strong self-etching adhesive systems to dentin.

Materials and Methods: One hundred twenty recently extracted, noncarious human molars were used to obtain superficial dentin substrate for bonding. No desensitizer was used in the control groups. The experimental groups were pretreated with Gluma Desensitizer, MicroPrime B, and Dentin Desensitizer immediately prior to bonding with self-etching adhesives Optibond XTR, Xeno IV, and iBond. A bonding jig was used to fabricate composite cylinders, which were stored for either 24 hours or three months, after which the shear bond strength (SBS) was evaluated using a notched-edge testing device at a crosshead speed of 1 mm/min. Failure mode distribution was also evaluated at 24 hours and three months. A two-way analysis of variance, Tukey test, and Student *t*-test, with a significance level of $p<0.05$, were used for data analysis.

Results: At 24 hours, there was no significant difference in SBS when the same adhesive was used with any of the experimental desensitizing agents compared with the control group without desensitizer. However, at three months, Dentin Desensitizer bonded with Optibond XTR demonstrated significantly lower SBS ($p<0.001$), while Gluma bonded with iBond showed significantly higher SBS values ($p=0.034$) relative to their corresponding control group. Only MicroPrime B bonded with Xeno IV and iBond with no desensitizer demonstrated a significant reduction in SBS after three months ($p=0.034$ and $p=0.002$, respectively). The most prevalent type of failure was adhesive.

Conclusion: Desensitizing agents can be used in combination with self-etching adhesives to control hypersensitivity without adversely affecting their bond strength to dentin.

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INTRODUCTION

Dentinal sensitivity is a painful condition that affects between 10% and 20% of the population.[1] Its etiology may involve tooth wear, gingival recession, tooth bleaching, and many restorative procedures. In particular, postoperative sensitivity is often observed as an undesirable outcome to the placement of resin composite restorations.[2,3] The prevalence of postoperative sensitivity following application of bonded resins has been reported to range between 0% and 50%.[4-6]

The two approaches used today in adhesive dentistry for the placement of resin composite restorations are etch-and-rinse and self-etch.[7] The former uses 32%-37% phosphoric acid etchant prior to infiltration with resin monomers, whereas the latter uses self-etching primers. While phosphoric acid dissolves the smear layer and opens dentin tubules for infiltration with resin monomers, self-etching primers partially dissolve hydroxyapatite, modifying the smear layer rather than dissolving it and thus becoming part of the hybrid layer.[8,9] In addition to being regarded as less technique sensitive, self-etching adhesives are also known to yield lower postoperative sensitivity compared with etch-and-rinse systems.[8] This is largely the result of the less aggressive demineralization pattern and thus the more superficial interaction with dentin, which leaves tubules largely obstructed with smear minimizing water movement across the interface.[8,10,11] However, postoperative sensitivity is still a relatively common finding with self-etching adhesives, perhaps because of the continued action of the acidic monomers, which causes further demineralization beyond the depth of adhesive resin infiltration, leaving areas of unencapsulated collagen at the bottom of the hybrid layer where fluid movement can still occur.[12]

Of the different theories put forward to explain the mechanism of dentin hypersensitivity, the most widely accepted is Brännström's hydrodynamic theory. A stimulus acts on the open tubules of the exposed dentin increasing the rate of dentinal fluid flow and generating action potentials in intradental nerves, which are passed on to the brain, generating pain.[13] Treatment strategies for dentin hypersensitivity may include the use of neural stimulus blockers, anti-inflammatory drugs, protein precipitants, tubule-occluding agents, and lasers.[14,15] The use of topically applied dentin desensitizers is a common and effective method to treat dentin hypersensitivity. Different agents have been used for this purpose, including oxalates, which create tubule obstruction by precipitating fine-grained calcium oxalate crystals[16-18]; dentin adhesives[19,20]; and protein-precipitating fixative agents.[21,22] Widely used desensitizing components include fluoride (Fluoridin N5, VOCO, PrepEze, Pentron Clinical, Orange, CA, USA), glutaraldehyde/hydroxyethyl methacrylate (HEMA; Gluma, Heraeus Kulzer GmbH, MicroPrime B, Danville Materials, San Ramon, CA, USA), and oxalate (BisBlock, Bisco, Schaumburg, IL, USA). According to Porto and others,[23] desensitizers containing glutaraldehyde/HEMA are considered the first treatment choice for dentinal hypersensitivity. While glutaraldehyde is a biological fixative known to cause coagulation of plasma proteins in the dentin fluid physically blocking dentinal tubules,[24] HEMA physically blocks the dentinal tubules.[25] Morphological and clinical studies with Gluma desensitizer, an aqueous solution of 5% glutaraldehyde (GA) and 35% HEMA, have shown peripheral tubular blockage and significant pain relief following topical application to hypersensitive dentin.[21,22] Moreover, Gluma desensitizer has also been shown to either maintain[26,27] or improve dentin bond strength.[28-30]

Restorative treatment is often indicated in conjunction with the use of desensitizing agents.[23] Moreover, the use of desensitizing agents has been incorporated, almost as a routine procedure, in most adhesive restorative procedures irrespective of the bonding approach.[31] However, the effect of desensitizers on bond strength is still controversial. A few studies have reported positive or no effect[27,29,32-35] while others have shown a negative effect[27,33,34] on the bond strength when desensitizing agents were incorporated into the bonding sequence. Despite the benefits associated with their use, the bond performance may be affected, compromising the integrity and longevity of adhesive restorations. Hence, evaluation of the effect on different desensitizing agents on the bond performance of various adhesive systems is needed to understand the benefit-risk ratio associated with their use when combined with different adhesive systems.

Hence, the objective of this *in vitro* study was to evaluate the effect of different desensitizing agents on the shear bond strength (SBS) of mild and strong self-etching adhesive systems to dentin. The null hypothesis was that the desensitizing agents would have no effect on the SBS to dentin of the different self-etching adhesive systems at 24 hours and three months.

METHODS AND MATERIALS

One hundred twenty recently extracted, noncarious human molars were used to obtain superficial dentin

Table 1: *Study Materials: Composition and Application Protocol as per the Manufacturer's Description*

Material	Manufacturer	Lot No.	pH	Composition	Application Protocol
Optibond XTR	Kerr Corp	3565224	2.5	Primer: Acetone (25%-35%), ethyl alcohol (4%-15%), HEMA (30%-50%) Adhesive: Ethyl alcohol (20%-30%), alkyl dimethacrylate resins (47%-68%), barium aluminoborosilicate glass (5%-15%), fumed silica (silicon dioxide; 3%-10%), sodium hexafluorosilicate (0.5%-3%)	• Apply primer and scrub for 20 s • Air thin for 5 s with medium air pressure • Apply adhesive and scrub for 15 s • Air thin for 5 s • Polymerize for 10 s
iBond	Heraeus Kulzer	010107	1.7	Acetone (25%-50%), 4-META (10%-25%)	• Apply iBond SE and scrub for 20 s • Carefully air dry for 5-10 s or until surface appears glossy • Polymerize for 20 s (with 400-500 mW/cm^2)
Xeno IV	DENTSPLY Caulk	100111	2.3	Acetone (<50%), UDMA (<15%), dipentaerythriotol pentaacrylate phosphate (<15%), polymerizable dimethacrylate resin (<10%), polymerizable trimethacrylate resin (<10%), polymerizable dimethacrylate resin (<10%)	• Apply XIV in two coats and scrub for 15 s • Gently air dry for 5 s • Polymerize for 10 s
Gluma Desensitizer	Heraeus Kulzer	010094	1.8	HEMA (25%-50%) Glutaraldehyde (5%-10%) Water	• Apply desensitizer and let it sit for 30-60 s • Dry surface until fluid film disappears • Apply adhesive resin
MicroPrime B	Danville Materials	17683	3.6	HEMA (25%-45%) BAC (1%-5%) Sodium fluoride (10 ppm)	• Apply desensitizer with microbrush and let it sit for 30 s • Air dry or leave moist • Apply adhesive resin
Dentin Desensitizer	Pulpdent	100721	5.6	Glutaraldehyde (5%) Sodium fluoride (1%) Water	• Apply desensitizer for 20-30 s • Blot or apply short blast of air to remove excess, but do not dry • Apply adhesive resin

Abbreviations: 4-META, 4-methacryloxyethyl trimellitic acid anhydride; BAC, benzalkonium chloride; HEMA, 2-hydroxyethyl methacrylate; UDMA, urethane dimethacrylate.[a]

substrate for bonding. The teeth were obtained under a protocol approved by the State University of New York's Institutional Review Board and stored in a 0.5% Chloramine-T solution at 4°C before use. The crowns were separated from the roots with a slow-speed diamond saw and embedded in a chemically polymerized methacrylate (Fastray, HJ Bosworth, Skokie, IL, USA) with the facial surface exposed and ground flat on a model trimmer to reveal superficial dentin, which was finished with 320-, 400-, and 600-grit silicon carbide abrasive paper (Buehler, Evanston, IL, USA). The specimens were stored in deionized water at 4°C until ready to be used. One hour prior to bonding, the specimens were acclimatized to room temperature (23 ± 2°C) and refinished with 600-grit abrasive paper to expose fresh dentin.

Self-etching adhesive systems, Optibond XTR (Kerr Corporation, Orange, CA, USA), iBond (Heraeus Kulzer GmbH, Hanau, Germany), and Xeno IV (DENTSPLY Caulk, Milford, DE, USA) were used in combination with the desensitizers Gluma Desensitizer (Heraeus Kulzer GmbH), MicroPrime B (Danville Materials), and Dentin Desensitizer (Pulpdent Corp, Watertown, MA, USA) for bond strength evaluation at 24 hours and three months. The composition and application protocol for all the materials evaluated in this study, as recommended by their manufacturer, are summarized in Table 1. Each adhesive system was used either with no desensitizer (control) or in combination with one of three desensitizers for a total of 12 groups with a sample size of 20 per group. Two hundred forty specimens were equally and randomly assigned to the 12 groups as follows: 1, Optibond XTR + Gluma; 2, Optibond XTR + MicroPrime B; 3, Optibond XTR + Dentin Desensitizer; 4, Optibond XTR alone (control); 5, iBond + Gluma; 6, iBond + MicroPrime B; 7, iBond + Dentin Desensitizer; 8, iBond alone (control); 9, Xeno IV + Gluma; 10, Xeno IV + MicroPrime B; 11, Xeno IV + Dentin Desensitizer; 12, Xeno IV alone (control).

After dentin pretreatment with or without desensitizer, the corresponding adhesives were applied

Table 2: *Mean (SD) Shear Bond Strength Results for the Different Combinations of Adhesive and Desensitizing Agent at 24 hours (n=10)*

	Gluma	MicroPrime B	Dentin Desensitizer	Control
Optibond XTR	34.5 (9.8)^{A,a}	44.2 (10.1)^{A,a}	35.4 (11.7)^{A,a}	39.1 (8.4)^{A,a}
iBond	34.9 (9.7)^{A,a}	29.4 (11.8)^{A,b}	21.7 (6.6)^{A,b}	33.7 (9.1)^{A,a*}
Xeno IV	36.9 (21.0)^{A,a}	41.8 (11.3)^{A,a,b*}	31.1 (12.2)^{A,a,b}	40.6 (15.0)^{A,a}

The same superscript letter indicates no significant differences among groups (Tukey test; p<0.05). Uppercase denotes differences among desensitizers for each adhesive (horizontal). Lowercase denotes differences among adhesives for each desensitizer (vertical)
** Groups that were significantly different from their corresponding three-month values (Student t-test; p<0.05).*

and polymerized, as per each of their manufacturer's instructions, with an LED light-curing unit (Bluephase 16i, power density 1600 mW/cm^2, Ivoclar-Vivadent, Amherst, NY, USA) in high-intensity mode. The specimens were stabilized on a bonding jig (Ultradent, South Jordan, UT, USA) with a cylindrical mold of standardized dimensions (2.38 mm in diameter and 2 mm in height). Composite cylinders were fabricated with resin composite (Filtek Z100, Lot No. N196007, 3M ESPE, Saint Paul, MN, USA) in shade A2 by application of only one increment no greater than 2 mm and polymerized for 20 seconds. The specimens were stored in distilled water containing 0.02% sodium azide at 37°C for either 24 h or three months, after which SBS was evaluated. A calibrated testing device (UltraTester, Ultradent) loaded at a crosshead speed of 1 mm/min and a load cell of 1000 lb (453.6 kg) was used. A notched-edge crosshead matching the diameter of the bonded cylinder was used to apply the testing load. The load required to debond the specimen was recorded and expressed in megapascals (MPa) and the group means calculated.

Individual two-way analysis of variance (ANOVA) tests were conducted to evaluate the effect of the main variables adhesive and desensitizing agent as well as their interactions on the bond strength at 24 hours and three months. A post hoc multiple-comparisons Tukey test was used for pairwise comparisons between group means. Student *t*-tests were used to evaluate differences between 24 hours and three months for each of the individual combinations. A significance level of $p<0.05$ was used for all tests. All statistical analyses were performed with SigmaStat version 3.5 (San Jose, CA, USA). Analysis of the failure mode was conducted through observations, by a single trained evaluator (Z.W.), with a field emission scanning electron microscope in secondary electron and backscattered modes (Hitachi SU-70, Hitachi, Krefeld, Germany) at a magnification of 50×. The failed interfaces were classified as adhesive (A), cohesive in dentin (D), cohesive in composite (C), or mixed (M).[36] Mixed failure was defined as the combination of different failure modes resulting from failure across the interfacial layers.

RESULTS

Two-way ANOVA revealed a significant effect of the variables, adhesive and desensitizing agent, on the bond strength both at 24 hours ($p=0.003$ and $p=0.016$) and three months ($p<0.001$ and $p<0.001$). The interaction between these variables was significant only at three months ($p=0.002$). The ranking of the groups, from highest to lowest SBS values, was the same whether it was evaluated at 24 hours or three months as follows: MicroPrime B > Gluma > Dentin Desensitizer and Optibond XTR > Xeno IV > iBond. Mean (SD) SBS values for the different combinations of adhesive and desensitizing agent at 24 hours and three months are summarized in Tables 2 and 3, respectively. In general, the use of desensitizing agents yielded no differences in SBS values relative to the control without desensitizer. Only two groups, when evaluated after three months, demonstrated significant variations from the control group. Dentin Desensitizer bonded with Optibond XTR showed significantly lower SBS values ($p<0.001$), and Gluma bonded with iBond showed significantly higher SBS values ($p=0.034$) relative to the control group (Table 3). MicroPrime B bonded with Optibond XTR demonstrated the highest mean SBS values of all groups both at 24 hours and three months (44.2 and 46.9 MPa, respectively). These values, however, were not significantly different from their corresponding control groups with no desensitizer.

Student *t*-test for each of the individual combinations of adhesive and desensitizing agent revealed no significant differences after three months of storage relative to their 24-hour values, with the exception of MicroPrime B bonded with Xeno IV and iBond without desensitizer, which demonstrated a significant decrease in SBS after three months ($p=0.034$ and $p=0.002$, respectively). The percentage bond variation after three months relative to 24 hours is also summarized in Table 3.

Table 3: *Mean (SD) Shear Bond Strength Results for the Different Combinations of Adhesive and Desensitizing Agent at Three Months (n=10)*

	Gluma	MicroPrime B	Dentin Desensitizer	Control
Optibond XTR	37.3 (13.8)^{A,B,a}	46.9 (5.5)^{A,a}	27.4 (9.4)^{B,a}	43.9 (7.7)^{A,a}
	8.1%	6.0%	−22.5%	12.1%
iBond	32.4 (10.2)^{A,a}	31.8 (9.4)^{A,B,b}	25.7 (7.7)^{A,B,a}	21.2 (6.3)^{B,b*}
	−7.1%	8.0%	18.4%	−37.1%
Xeno IV	29.4 (9.7)^{A,a}	31.4 (8.7)^{A,b*}	27.7 (9.4)^{A,a}	34.9 (8.7)^{A,a}
	−20.4%	−24.8%	−10.8%	−13.9%

Percentage bond variation after three months relative to 24 hours. Positive percent values denote bond strength increase. Negative percent values denote bond strength decrease. The same superscript letter indicates no significant differences among groups (Tukey test; p<0.05). Uppercase denotes differences among desensitizers for each adhesive (horizontal). Lowercase denotes differences among adhesives for each desensitizer (vertical).
** Groups that were significantly different from their corresponding 24-hour values (Student t-test; p<0.05).*

The failure mode distribution for specimens fractured at 24 hours and three months is summarized in Figures 1 and 2, respectively. Overall, the most prevalent failure mode was adhesive in nature. Relative to 24 hours, the number of adhesive failures increased for all groups after three months. Cohesive fractures in dentin were also observed, particularly at 24 hours, but were fewer in number. A few cohesive fractures in composite were shown at 24 hours only but not after three months. No correlations were observed between bond strength values and mode of failure.

DISCUSSION

The present study investigated the effect of different desensitizing agents on the SBS of mild and strong self-etching adhesives. The null hypothesis was only partially rejected as the desensitizing agents generally showed no effect on the SBS to dentin of the different self-etching adhesive systems at 24 hours or three months. Only two exceptions were observed at three months: Dentin Desensitizer bonded with Optibond XTR, which showed lower SBS than the control, and Gluma bonded with iBond, which showed higher SBS than the control. Our results are in agreement with previous studies,[26,27] which have shown that desensitizing agents can be safely incorporated into the bonding sequence without adversely affecting the bond performance. Despite the lack of significant variations relative to their corresponding control group, certain combinations seemed more favorable than others. Optibond XTR demonstrated the best performance of the three adhesives. After three months, when Optibond XTR was used either alone or in combination with Gluma or MicroPrime B, an increase in bond strength was shown. We speculate that this may have been the

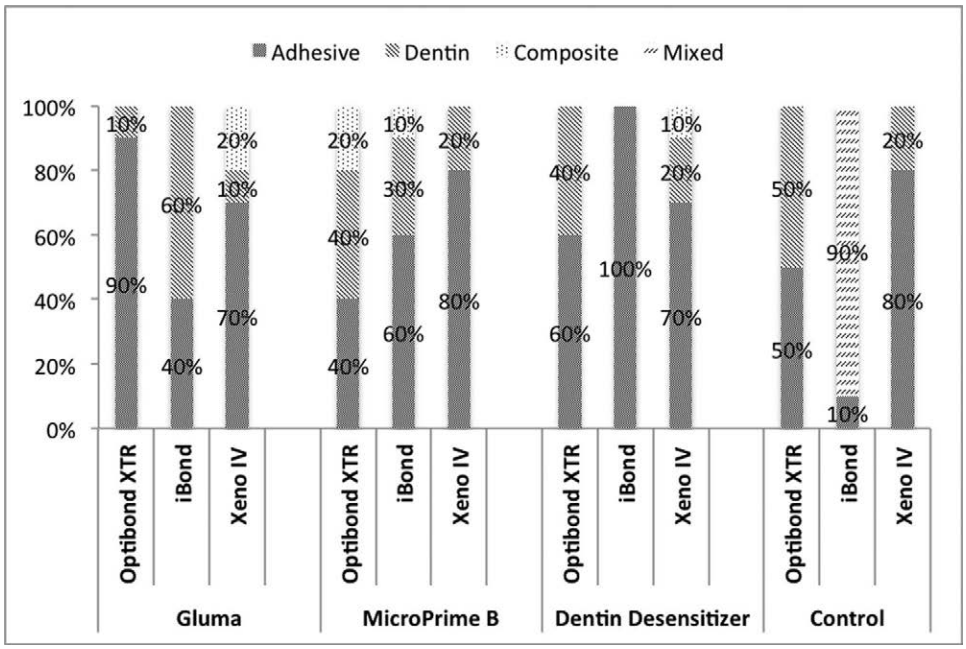

Figure 1. *Failure mode distribution for the different combinations of adhesive and desensitizing agent at 24 hours (n=10). Modes of failure described as adhesive (A), cohesive in dentin (D), cohesive in composite (C), and mixed (M).*

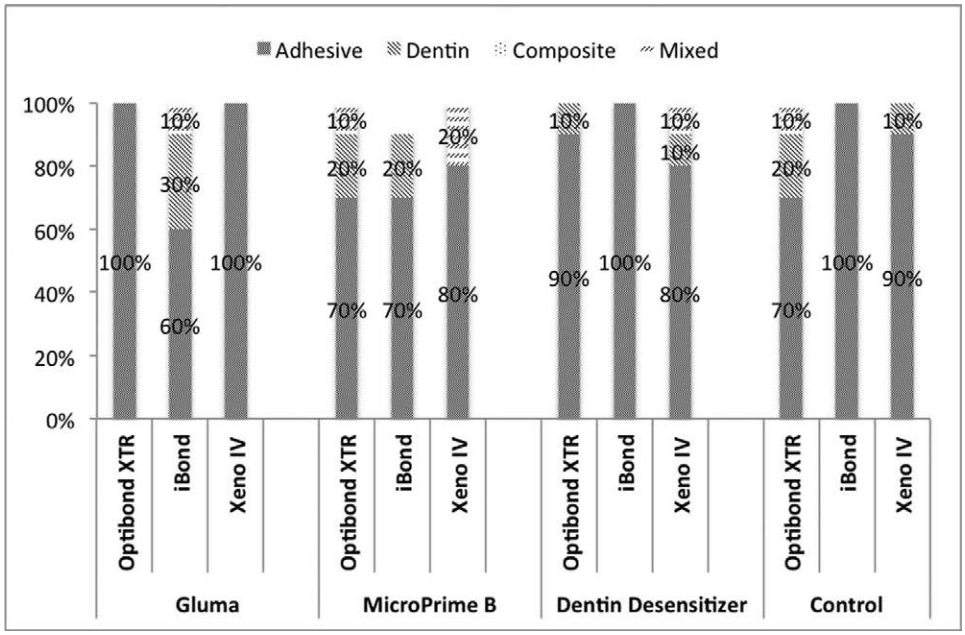

Figure 2. *Failure mode distribution for the different combinations of adhesive and desensitizing agent at three months (n=10). Modes of failure described as adhesive (A), cohesive in dentin (D), cohesive in composite (C), and mixed (M).*

result of compatibility aspects between materials, as well as their individual composition and pH. Optibond XTR film thickness is in the range of 5 to 10 μm. Its reduced film thickness, which improves its wetting properties, in combination with its hydrophilic nature, which keeps collagen from collapsing, may have contributed to a greater monomer infiltration and consequently to a longer resin tag formation.

Three self-etching adhesives were used in this study, which were different in composition, pH, and their bonding approach. While Optibond XTR is a two-step system, including the self-etching primer, and mostly solvent free, the hydrophobic adhesive resins, iBond and Xeno IV, are all-in-one systems, combining acidic, hydrophilic, and hydrophobic monomers with organic solvents and water into a single bottle. All-in-one systems are known to form highly hydrophilic polymers that behave as permeable membranes, allowing diffusion of water from dentin across the hybrid and adhesive layers.[37] This may help explain the overall superior performance of the two-step system, Optibond XTR, relative to all-in-one adhesives, Xeno IV and iBond. In general, self-etching adhesives can be classified as ultra-mild (pH>2.5), yielding demineralization of only a few hundreds of nanometers; mild (pH~2), with demineralization of about 1 μm; intermediately strong (pH=1-2), with demineralization about 1-2 μm; and strong (pH≤1), with demineralization greater than 2 μm. Only with intermediately strong and strong self-etching adhesives can typical resin tags be formed, while they are hardly formed with mild and ultra-mild self-etching adhesives.[38] The interfacial structure of highly acidic self-etching adhesives resembles that of etch-and-rinse systems, with the difference that the dissolved mineral phase is not rinsed away. These calcium phosphates are known to be very unstable, greatly contributing to weakening the interface.[38] Moreover, as the acidity of the adhesive increases, the issues with water permeability also become more acute, leaving water-filled nano-spaces at the interfacial layer. Water contributes to both degradation of collagen fibrils and composite plasticization, leading to the accelerated destruction of the hybrid layer and the consequent loss of the dentin bond strength over time.[38] Of the adhesives evaluated in our study, Optibond XTR (pH 2.5) and Xeno IV (pH 2.3) can be considered ultra-mild and mild, respectively, whereas iBond can be considered intermediately strong. The greater acidity of iBond may help explain its overall lower performance relative to Xeno IV and Optibond XTR. Only when iBond was bonded with Gluma and evaluated after three months was a significantly higher bond strength relative to the control group seen.

Because of the water content in these mixtures, desensitizing agents also serve as rewetting agents that expand the demineralized collagen network and increase its surface energy, all of which facilitate the diffusion of resin monomers into the partially demineralized dentin, thus improving resin-dentin bonds.[39] Hydroxyethyl methacrylate, a water-soluble monomer present in most dental adhesives and

in two of the three desensitizers evaluated, is known to improve infiltration of the partially demineralized collagen because of its ester and hydroxyl functional groups and its hydrophilic nature.[40] Because of its known role in facilitating diffusion of resin monomers into the partially demineralized collagen network, HEMA is considered an adhesion promoter.[41] Moreover, strong inhibitory properties of matrix metalloproteinase MMP–2[42] and MMP-9[43] have also been shown with monomeric HEMA. However, the frequently reported fall in bond strength of HEMA-containing adhesives[44,45] indicates that the MMP-inhibitory effects of HEMA may be lost when it is copolymerized with other adhesive monomers.[43] Overall, Dentin Desensitizer, the only HEMA-free desensitizer evaluated in our study, demonstrated lower performance than HEMA-containing desensitizers, Gluma and Micro-Prime B. Of the three desensitizers evaluated, MicroPrime B demonstrated the best overall performance when the data were combined. When the data were analyzed separately at 24 hours and three months, MicroPrime B was also shown to perform best, particularly when combined with Optibond XTR and Xeno IV. MicroPrime B contains benzalkonium chloride (BAC) and sodium fluoride in addition to HEMA. As a surface surfactant, BAC may allow for a more interactive surface. Conversely, the desensitizing mechanism of sodium fluoride is derived from the formation of precipitates, which mechanically block the exposed dentinal tubules.[46] Studies have shown a reverse relation between the amount of fluoride in the desensitizing agent and dentin bond strength.[47] This may be the result of crystal precipitation, which may physically prevent penetration of the adhesive resin monomers.[48,49] Availability of calcium ions on the dentin surface is critical to the formation of precipitates, suggesting that less precipitation will occur in a more acidic environment. Moreover, fluoride salts are soluble at lower pH values, making crystal precipitates more likely to remain intact when in a mildly acidic environment such as the one created by Dentin Desensitizer (pH=5.6) and to a lesser extent MicroPrime B (pH=3.6). Application of a highly acidic overlaying self-etching adhesive may further dissolve these precipitates.

The common active component of Gluma and Dentin Desensitizer is 5.0% glutaraldehyde. Of the two agents, Gluma demonstrated better overall performance. Gluma has been used since 1991 as a dentin desensitizer.[50] Its desensitizing mechanism remained elusive until Schüpbach and others showed that topical application *in vivo* of glutaraldehyde/HEMA combination products resulted in a series of horizontal partitions within the lumens of exposed dentinal tubules.[22] More recent spectrophometric studies *in vitro*[25] revealed that the glutaraldehyde in Gluma reacts with plasma proteins such as albumin to form protein precipitates, which then react with HEMA to form a mixture of poly-HEMA copolymerized with glutaraldehyde–cross-linked albumin.[51] These precipitates occlude open dentinal tubules beneath the surface, which interferes with the hydrodynamics of dentinal fluid, thereby preventing dentin sensitivity. It remains unclear how long these precipitates stay in dentinal tubules. Dentinal fluid and saliva contain esterases[52] that could attack the ester and peptide bonds in these intratubular precipitates.[53] Saliva also contains MMPs[54] and kallikreins[55] that could attack collagen and plasminogen, respectively. If these enzymes attacked the Gluma-created precipitates, their desensitizing activity would be lost if the intratubular precipitates were destroyed. Glutaraldehyde is also a potent antimicrobial and cross-linking agent known to improve the resistance of un–cross-linked or mildly cross-linked collagen matrices to enzymatic degradation by collagenases.[56,57] The mechanism seems to be dependent on the reaction between the aldehyde group of glutaraldehyde and the amino groups of lysine and hydroxylysine residues in collagen[58,59] that increase the resistance of collagen to enzymatic degradation.[59] By improving dentin's mechanical properties, glutaraldehyde can also minimize the degradation of the resin-dentin bonds.

In general, no significant differences were shown among adhesives, desensitizers, or after storage, so speculations have been provided based on trends only. Although it was not the intent of our study to evaluate the degradation patterns of the interfaces created with different combinations of materials, the drop in bond strength values observed for some groups after only three months was unexpected and caused concern. Future bond degradation studies are thus necessary to confirm these trends. Only MicroPrime B bonded with Xeno IV and iBond without desensitizer demonstrated a significant bond strength reduction after three months, with a 24.8% and 37.1% drop, respectively. Groups such as Dentin Desensitizer bonded with Optibond XTR and Gluma bonded with Xeno IV, with a drop in bond strength values of 22.5% and 20.4%, respectively, although not significantly different from their corresponding 24-hour values, were still of concern. The positive percentage variation values

in Table 3 indicate groups whose values increased after three months. Although these differences were not significant, they may indicate further maturation of the bonds. A postirradiation polymerization reaction takes place after initial irradiation, and it is known to extend to the first 24 hours.[60] Increased bond strength values, however, may suggest further maturation of the bonds past the initial 24 hours. Since the acidity of self-etching adhesives continues to demineralize the dentin matrix beneath the hybridized layer,[61] we speculate that further diffusion into the matrix and chemical bonding of some resin monomers in these groups may have continued to strengthen the hybrid zone.

Although our results are limited to the systems and techniques evaluated in this study and cannot be extrapolated to draw conclusions as to the behavior of self-etching adhesives when used in combination with different desensitizing agents, they suggest the use of desensitizing agents does not affect initial bond performance. Future bond degradation studies with a larger sample size and longer incubation times are needed to identify differences in degradation patters when using different combinations of adhesives and desensitizing agents. Moreover, micro-tensile bond strength tests may be recommended for bond degradation studies since long incubation periods, between 2 and 4 years, may be necessary to detect the effects of hydrolytic degradation for specimens of a large surface area such as those used in SBS tests due to the required longer diffusional distances from the cavosurface margin.[62] When compared with microtensile tests, SBS tests are also known to be less discriminating in their ability to detect differences, perhaps requiring a considerably larger sample size to be able to detect differences. One of the limitations of the present study may have been the sample size of 10, which may have limited our ability to detect differences. Further evaluation of different combinations of adhesives and desensitizing agents, as well as evaluation of desensitizers with different mechanisms of action, should precede the adoption of clinical techniques involving incorporation of desensitizing agents in the bonding sequence.

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that the effect of desensitizing agents on the SBS of self-etching adhesives to dentin appears to be material and time dependent. This indicates that they may be safely incorporated in the bonding sequence of self-etching systems without adversely affecting their bond performance.

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Conflict of Interest

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

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Faculty Positions

Full-Time Non-Tenure Track Faculty Position
UCLA School of Dentistry

The UCLA School of Dentistry invites applications for a full-time, non-tenure track faculty position at the level of Health Sciences Assistant or Associate Clinical Professor in the Section of Restorative Dentistry, Division of Constitutive and Regenerative Sciences. This position is available starting January 1, 2016, and the search will remain open until the position is filled.

Primary responsibilities of the position include teaching in the Section of Restorative Dentistry, which includes but is not limited to the predoctoral curriculum at both clinical and preclinical levels. Background or expertise in esthetic dentistry as well as strong records of clinical teaching are preferred, but not required.

Applicants must possess a DDS or equivalent degree from the CODA-approved dental school; and/or a certificate in Prosthodontics from a CODA approved program. Applicants must possess or be eligible for a California dental license. An opportunity for part-time intramural clinical practice will be available. Demonstration of commitment to innovative, scholarly research, teaching excellence, and outstanding patient care is highly desirable, as well as demonstration or likely commitment to diversity-related teaching/research/service. Salary is commensurate with education and experience.

Applications will be accepted until the position is filled. Applicants should submit a cover letter, curriculum vitae, research plan, teaching statement, and the names of three references to Steven Shaevel, Academic Personnel Director, via UCLA Recruit https://recruit.apo.ucla.edu/apply/JPF01464.

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Any questions, please contact Jill Uebelhoer in Restorative Dentistry at (716) 829-2862 or e-mail at: jgu@buffalo.edu.

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Director of Biomaterials
Faculty Position F39220
VCU School of Dentistry, Department of General Practice

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Required qualifications include: DDS/DMD degree, strong leadership skills, and documented experience in the areas of dental practice or clinical teaching.

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For more information, contact **Dr. Alfred (Fred) Certosimo, Chair of Search Committee, Department of General Practice, School of Dentistry, Virginia Commonwealth University, PO Box 980566, Richmond, VA 23298.**

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Microshear Bond Strength of Adhesives to Enamel Remineralized Using Casein Phosphopeptide Agents

EH Mobarak • N Ali • LE Daifalla

Clinical Relevance: Remineralization of enamel with casein phosphopeptide—amorphous calcium phosphate with fluoride had no negative effect on its bonding to self-etch adhesives. The newly developed Single Bond Universal adhesive system did not achieve better bonding to enamel than did its predecessors.

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Lesion Activity Assessment (LAA) in Conjunction With International Caries Detection and Assessment System (ICDAS) for Occlusal Caries Diagnosis in Permanent Teeth

FVMD Cotta • LS de Castilho • AN Moreira • SM Paiva • EF Ferreira • LCN Ferreira • CS Magalhães

Clinical Relevance: The ICDAS proved to be a reproducible method with good performance in detecting carious lesions in the dentin threshold. The ICDAS-LAA criteria were reproducible to assess caries activity, but with a low degree of accuracy.

doi: http://dx.doi.org/10.2341/13-332-C

Evaluation of the Radiopacities of Bulk-fill Restoratives Using Two Digital Radiography Systems

E Yasa • B Yasa • OS Aglarci • ET Ertas

Clinical Relevance: Bulk-fill restoratives had higher radiopacity values than dentin and enamel at varying thicknesses, which makes these restoratives suitable for radiographic visualization of caries.

doi: http://dx.doi.org/10.2341/14-074-L

Effect of Photoactivation Timing on the Mechanical Properties of Resin Cements and Bond Strength of Fiberglass Post to Root Dentin

RD Pereira • ADCM Valdívia • AA Bicalho • SD Franco • D Tantbirojn • A Versluis • CJ Soares

Clinical Relevance: Delayed photo-activation may be beneficial for the clinical behavior of resin cements used to lute fiber posts. The root canal region is still a critical factor for the mechanical behavior of dual-cure resin cements.

doi: http://dx.doi.org/10.2341/14-115-L

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Microshear Bond Strength of Adhesives to Enamel Remineralized Using Casein Phosphopeptide Agents

EH Mobarak • N Ali • LE Daifalla

Clinical Relevance

Remineralization of enamel with casein phosphopeptide—amorphous calcium phosphate with fluoride had no negative effect on its bonding to self-etch adhesives. The newly developed Single Bond Universal adhesive system did not achieve better bonding to enamel than did its predecessors.

SUMMARY

Objective: This study was carried out to evaluate the difference between bonding to demineralized enamel and remineralized enamel using casein phosphopeptide–amorphous calcium phosphate with fluoride (CPP-ACFP) or without fluoride (CPP-ACP) compared to normal enamel. Another aim was to test if the newly introduced Single Bond Universal adhesive system would show better bonding to any enamel condition in comparison to the other tested adhesive systems.

Methods: The lingual enamel surfaces of 40 non carious human third molars were divided into four main groups according to the enamel condition (ground normal enamel [negative control]; demineralized enamel [positive control]; and remineralized enamel with CPP-ACP or with CPP-ACFP, respectively). Within each main group, the lingual enamel surface of each tooth was sectioned into three slabs, resulting in 30 slabs that were distributed into three subgroups according to the adhesive system utilized (Clearfil S^3 Bond Plus, Single Bond Universal, or G-aenial Bond). Two resin composite microcylinder buildups were made on each enamel slab using Filtek Z350 XT. The μSBS was evaluated at a crosshead speed of 0.5 mm/min. Modes of failure were detected using an environmental scanning electron microscope at 300× magnification.

Results: The two-way analysis of variance with repeated measures revealed a significant effect for the enamel condition. However, there was no significant effect for the type of

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adhesive system. The interaction between the enamel condition and the type of adhesive system was also not significant. Modes of failure were mainly adhesive except for the demineralized enamel. It showed a mixed type of failure, in which cohesive failure in enamel was recorded.

Conclusions: All single-step self-etch adhesives revealed comparable μSBS values to ground enamel and enamel remineralized with CPP-ACP or CPP-ACFP. Bonding to demineralized enamel was ineffective. With any enamel condition, no tested single-step self-etch adhesive was superior in its bonding.

INTRODUCTION

Scientific advances in restorative materials and techniques as well as in understanding the pathogenesis of caries and methods of its prevention have led to the evolution of minimal intervention dentistry. In this concept, healing of early subsurface lesions by remineralization is preferred to surgical intervention.

For enamel subsurface lesion remineralization, multiple approaches have evolved, including different fluoride regimens and calcium phosphate systems.[1] Previous investigations[2-8] have demonstrated the ability of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) to control demineralization and enhance remineralization. Casein phosphopeptides are thought to stabilize calcium and inorganic ions. Thus, they provide a reservoir of small CPP-ACP clusters with respect to the enamel surface controlling demineralization and enhancing remineralization The inclusion of fluoride into CPP-ACP resulted in a novel CPP-ACFP material that was suggested to reveal better remineralizing potential than CPP-ACP.[9] The availability of fluoride ions in conjunction with calcium and phosphate ions at the enamel surface was expected to enhance fluorapatite formation. In addition, CPP-ACFP was found to be superior in reducing caries risk, as compared to the products that contain only fluoride "fluoride *per se* products,"[10] in which the enamel has been shown to be more acid resistant.[11] Nevertheless, this acid-resistant enamel substrate might become an obstacle for bonding. Therefore, to clarify this issue, some studies[12-14] have tested the effect of remineralization with CPP-ACP on enamel bonding. However, at present there are no data available on the effect of remineralization with CPP-ACFP on bonding to enamel.

At the same time, the self-etch approach provides dentists with a generation of user-friendly and less technique sensitive adhesives that have paved the way for increasing its usage by general practitioners. The development of self-etch adhesives has continued in recent years and resulted in what is called "universal adhesive." The manufacturer claims that this adhesive system can be used with both direct and indirect restorations. It was also assumed that this adhesive system bonds effectively to all tooth substrates, including enamel and dentin, as well as to glass ceramic, zirconia, noble and nonprecious alloys, and composites without the need for an additional primer. However, there is a lack of information about the bond of this adhesive to enamel compared with other single-step self-etch adhesives. Therefore, a study to determine the microshear bond strength of this new self-etch adhesive system to different enamel conditions (ground enamel with no treatment, demineralized enamel, and enamel remineralized with CPP-ACP or with CPP-ACFP) could be of value. The null hypotheses were the following: 1) The μSBS values of CPP-ACP and CPP-ACFP remineralized enamel specimens are not different from each other or from those of the sound enamel group; and 2) There is no difference in bonding of the newly introduced single-step self-etch adhesive system and that of other adhesives, regardless of the enamel condition.

METHODS AND MATERIALS

Selection of Teeth

A total of 40 normal human third molars, free from previous restoration or decay and with sufficient enamel width lingually, were selected for this study. Teeth were collected from patients within the 20 to 25-year-old age group. Immediately after extraction, the teeth were thoroughly washed, scrubbed, and scaled to remove blood, mucous, and shreds of periodontal ligament. The study was accomplished in accordance with local human subjects' oversight committee guidelines. All teeth were examined using a (6×) magnifying lens (Bausch and Lomb, Opt. Co, Rochester, NY, USA) to ensure that they were free from visible hypoplastic defects or fractures. Teeth were stored refrigerated at 4°C in a phosphate buffer saline solution containing 0.2% sodium azide (pH=7.4) for a period not longer than one month.[15]

Grouping for the Study

The selected 40 teeth were sectioned at the level of the cemento-enamel junction to separate the crown portion and were then divided into four main groups.

Table 1: *Materials Used in the Study*

Material Brand Name	Composition	Manufacturer	Batch No.
Tooth Mousse	Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, zinc oxide, sodium saccharin, ethyl *p*-hydroxybenzoate, magnesium oxide, guar gum, propyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate	GC Corporation, Itabashi-Ku, Tokyo, Japan	300788TP
MI Paste Plus	Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, sodium fluoride, flavoring, sodium saccharin, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate	GC Corporation, Itabashi-Ku, Tokyo, Japan	300783TP
Clearfil S^3 Bond Plus	MDP, Bis-GMA, 2 HEMA, hydrophilic aliphatic dimethacrylate, hydrophobic aliphatic methacrylate, colloidal silica, sodium fluoride, DL camphorquinone, accelerators, initiator, ethanol, water, pH = 2.7	Kuraray Medical Inc, Sakazu, Okayama, Japan	00018A
G-aenial Bond	Acetone, distilled water, dimethacrylate, 4-META, anhydride, phosphoric acid ester monomer, silicon dioxide, photo initiator, pH = 1.5	GC Corporation, Itabashi-Ku, Tokyo, Japan	1103231
Single Bond Universal	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond™ copolymer, filler, ethanol, water, initiators, silane, pH = 2	3M ESPE Dental Products, St Paul, MN, USA	468355
Filtek Z350 XT	Bis-GMA, TEGDMA, PEGDMA, Bis-EMA, UDMA (zirconia-silica) 63.3% by volume and 78.5% by weight, filler particle size range (4-20 nm), cluster particle size (0.6-20 μm)	3M ESPE Dental Products, St Paul, MN, USA	N286859

Abbreviations: Bis-EMA, ethoxylated bisphenol A dimethacrylate; Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; CMC-Na, sodium carboxymethyl cellulose; CPP-ACP, casein phosphopeptide–amorphous calcium phosphate; 4-META, 4-methacryloxyethyl trimellitic acid; HEMA, 2-hydroxyethyl methacrylate; PEGDMA, polyethylene glycol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UDMA, urethane dimethacrylate.

In the first group, bonding was done to ground enamel with no pretreatment; this group served as the control. In the second group, bonding was done to demineralized enamel only. In the third and fourth groups, bonding was done to remineralized enamel with CPP-ACP or with CPP-ACFP, respectively. Then the lingual enamel surface of each coronal portion was vertically sectioned into three slabs, resulting in a total of 30 enamel slabs within each main group. The 30 slabs from each group were distributed equally into three subgroups (10 enamel slabs each) according to the adhesive system utilized. For the first subgroup, Clearfil S^3 Bond Plus was used. For the second and third subgroups, Single Bond Universal and G-aenial Bond adhesive systems were applied, respectively. The brand name, composition, manufacturer, and batch number of the materials used in the present study are listed in Table 1.

Preparation of Enamel Specimens

In order to standardize the position of the slabs during the embedding, a circle of 21 mm in diameter and two perpendicular intersecting lines were drawn on a square-shaped glass piece (50 mm × 50 mm) (Figure 1A). Each enamel slab was fixed from its lingual surface to the center of the circle using glue (Rocket Light, Dental Ventures of America, Corona, CA, USA). Polyvinyl chloride (PVC) tubes of 15-mm internal diameter and 21-mm external diameter were cut into two heights: 1-mm ring and 20-mm cylinder. The first PVC ring of 1-mm height (Figure 1B) was coated with a separating medium and placed on the glass piece coinciding with the drawn circle in order to raise the specimen above the embedding material by 1 mm. The second cylindrical PVC tube of 20-mm height was sealed from one end with an adhesive tape to serve as a mold (Figure 1C). The glass piece with the fixed enamel slab and the encircling 1-mm ring was then placed onto the PVC mold with the drawn circle coinciding with the mold outer margin to centralize the slab within the mold. The polyester embedding material (polyester #2121, ETERNAL CHEMICAL CO, LTD, Hsien, Taiwan) was mixed and poured to fill the mold. After complete setting of the embedding material, the glass slab and the 1-mm ring were removed, at which point the enamel slab was centrally embedded within the mold, while the specimen was protruding by 1 mm above the polyester embedding material. Each embedded enamel specimen was coded according to the enamel condition and the adhesive system

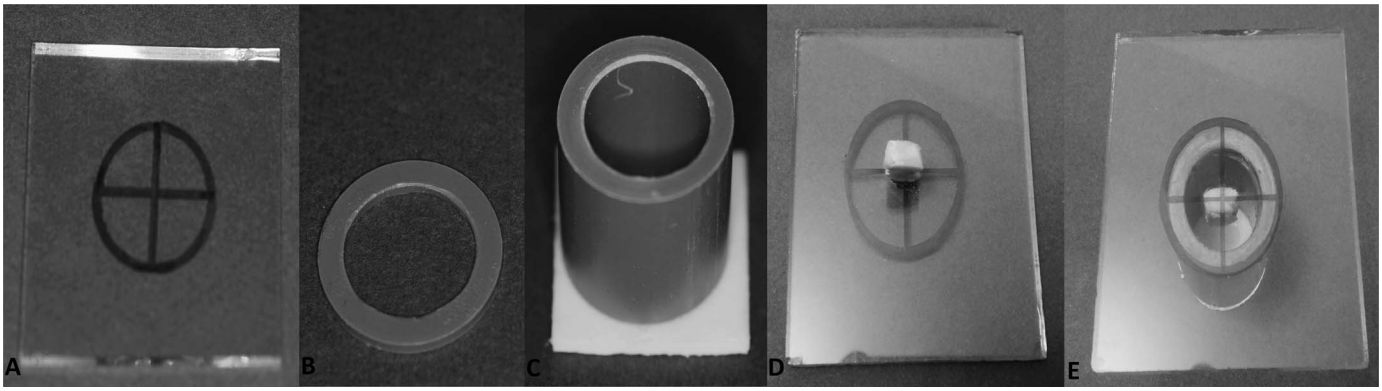

Figure 1. *Steps for embedding of the specimens: two perpendicular intersecting lines drawn on a square-shaped glass piece to standardize the position of the slabs during the embedding (A), 1-mm height PVC ring used to protrude the specimen above the embedding material (B), 20-mm height PVC cylindrical tube sealed from one end to serve as a mold (C). The enamel slab fixed on the glass piece (D). The glass piece with the fixed enamel slab and the encircling 1-mm ring placed onto the PVC mold (E).*

utilized. The enamel surface of each embedded specimen was then ground flat while ensuring that only 0.4 mm was removed from the total height of the specimen using a digital caliper (Proficraft, Mebschibes, Germany). The enamel surface was then finished manually with wet 600-grit silicon carbide waterproof abrasive paper (Grinco Miami, FL, USA) to ensure a uniform smear layer formation.[16]

Preparation for Different Enamel Conditions

Artificial caries-like lesions in the enamel were created by immersing each embedded specimen in 40 mL of demineralizing solution. The solution contained 0.1 mol/L lactic acid, 500 mg/L hydroxyapatite, and 20 g/L Carbopol C907 at pH 4.8.[17] Specially constructed plastic containers were made to hold the embedded enamel specimens during the demineralization and remineralization periods. The embedded specimens were held upside down in the demineralizing solution. The demineralization was done over the course of four days at 37°C, and the solution was changed every two days. After demineralization, specimens were removed and washed with distilled water for 30 seconds, then dried for 10 seconds.

For remineralization, each specimen was subjected to 2 mL of any of the proposed remineralizing solutions (2% CPP-ACP or CPP-ACFP) at 37°C, with daily change of the solution[18] over the course of 30 days. The remineralizing solution was prepared by dissolving 1 g of the remineralizing paste in 4 mL of water, resulting in 160 mL of remineralizing solution from each tube. A specially cut PVC tube (15 mm in length) was glued to the mold to enclose the remineralizing solution. The specimens were held upright during remineralization in the specially constructed plastic containers. Wet cotton was laid at the bottom of the container to maintain the humidity during the remineralization regimen. After the remineralization period, each specimen was removed and washed with 10 mL distilled water for 60 seconds.[18]

Restorative Procedures

The tested adhesive systems were applied to the enamel surfaces using disposable microbrushes (Tokuyama Dental America, Encinitas, CA, USA) according to the manufacturer's instructions, as described in Table 2. A transparent polyethylene tube obtained from a scalp vein infusion set (23G,

Table 2: *Steps of Application for the Adhesive Systems Utilized in the Study*

Adhesive System	Mode of Application
Clearfil S^3 Bond Plus	• Apply bond with a disposable microbrush • Leave in place for 10 s • Dry with mild pressure air flow for five s at 10-mm distance • Light-cure for 10 s
G-aenial Bond	• Apply with a disposable microbrush • Wait 10 s • Dry with oil-free air under maximum air pressure for five s at 10-mm distance • Light-cure for 10 s
Single Bond Universal	• Apply using disposable applicator and rub it for 20 s • Direct gentle steam of air for about five s at 10-mm distance until the film no longer moves • Light-cure for 10 s

JMS Singapore PTE, LTD, Singapore) was cut into small irises of 0.7 mm in length using a sharp lancet. The transparent polyethylene iris was used to assist in packing of the resin composite.[19] The external and internal diameters of the polyethylene iris were measured using a scanning electron microscope (515; Philips, Einhoven, The Netherlands) and were verified to be 2.35 mm for the external diameter and 0.93 mm for the internal diameter. Two resin composite microcylinders were built over each enamel specimen. Each resin composite microcylinder was polymerized for 40 seconds using a Blue Phase C5 light-curing unit (Ivoclar Vivadent, Schaan, Liechtenstein). The light intensity was regularly checked using an LED Radiometer (Kerr Dental Specialties, West Collins Orange, USA). The polyethylene irises were then cautiously removed with the aid of a No. 11 lancet (Wuxi Xinda Medical Device Co, China), leaving the composite microcylinders bonded to enamel surfaces. All resin composite microcylinders were checked using a (6×) magnifying glass lens for any defect or air bubble. Any defective microcylinders were excluded, along with their related slabs.

Microshear Bond Strength Assessment

Each specimen with its two bonded resin composite microcylinders was secured with the four tightening bolts in the lower part of the specially designed attachment jig.[20] The attachment jig was in turn screwed into the lower fixed and the upper movable compartments of the testing machine (Model LRX-plus; Lloyd Instruments Ltd, Ferham, UK). A shear load was applied via the testing machine at a crosshead speed of 0.5 mm/min. Data were recorded using computer software (Nexygen-MT; Lloyd Instruments). The bond strength of the resin composite microcylinders that showed spontaneous interfacial debonding during the handling or the mounting of specimens was recorded as 0 MPa.

Statistical Analysis

Two-way analysis of variance (ANOVA) with repeated measures was used to compare the effect of the enamel condition, the adhesive system, and their interaction. This was followed by the Tukey post hoc test for pairwise comparison. The significance level was set at $\alpha=0.05$. Data were analyzed using the SPSS program for Windows (release 15 for MS Windows, SPSS Inc, Chicago, IL, USA).

Failure Mode Examination

After measuring the bond strength, each fractured specimen was inspected using an environmental scanning electron microscope (ESEM) (Quanta 200, FEI Company, Philips) at 25 KV to determine its mode of failure. The failure modes were allocated to five types, as follows:

1) Type A: Adhesive failure (at the adhesive/enamel interface);
2) Type B: Cohesive failure in the adhesive;
3) Type C: Mixed failure including partial adhesive failure at the adhesive/enamel interface and partial cohesive failure in the adhesive;
4) Type D: Mixed failure including partial cohesive failure in the adhesive system and partial cohesive failure in resin composite; and
5) Type E: Mixed failure including adhesive failure at the adhesive/enamel interface, cohesive failure in enamel, cohesive failure in the adhesive, and cohesive failure in resin composite.

Representative photomicrographs for the most predominant types of failure within each subgroup were captured at 300× magnification.

RESULTS

The μSBS (MPa) results were described in terms of the mean standard deviation (SD). The two-way ANOVA revealed a significant effect for the enamel condition ($p<0.001$), while the type of adhesive system had no significant effect ($p=0.155$). The interaction between the enamel conditions and the type of adhesive system was also not significant ($p=0.700$). The effects of different enamel conditions on the μSBS values of the three tested single-step self-etch adhesive systems are presented in Table 3.

The distribution of failure modes for all tested groups are shown in Figure 2. The ESEMs revealed that the predominant mode of failure for the control enamel groups of the three tested adhesive systems was adhesive failure at the adhesive/enamel interface (type A). For the demineralized enamel groups, all tested adhesive systems showed type E mixed failure that includes adhesive failure at the adhesive/enamel interface, cohesive failure in enamel, cohesive failure in the adhesive, and cohesive failure in resin composite. With regard to bonding to the remineralized groups with CPP-ACP or CPP-ACFP, Clearfil S^3 Bond Plus and Single Bond Universal adhesive systems revealed type A as the predominant failure mode. For G-aenial Bond bonded to the remineralized group with CPP-ACP, type C mixed

Table 3: *The Effect of Different Enamel Conditions on the μSBS (MPa) of the Tested Adhesive Systems, Mean (Standard Deviation)*[a]

Adhesive Systems	Enamel Conditions, MPa				*p*-Value
	Control	Demineralized	Remineralized with CPP-ACP	Remineralized with CPP-ACFP	
Clearfil S^3 Bond Plus	11 (2.8) aA (Ptf/tnt=0/20)	4.1 (4.3) aB (Ptf/tnt=7/20)	10.1 (2.9) aA (Ptf/tnt=0/20)	10.9 (4.0) aA (Ptf/tnt=1/20)	<0.001
G-aenial Bond	11.7 (4.0) aA (Ptf/tnt=1/20)	2.1 (2.0) aB (Ptf/tnt=8/20)	10.5 (3.2) aA (Ptf/tnt=1/20)	10.8 (3.3) aA (Ptf/tnt=2/20)	<0.001
Single Bond Universal	13.9 (5.3) aA (Ptf/tnt=0/20)	3.2 (3.3) aB (Ptf/tnt=7/20)	13.0 (4.0) aA (Ptf/tnt=0/20)	11.0 (4.1) aA (Ptf/tnt=0/20)	<0.001

[a] *(ptf/tnt=pretest failure/total number of tested resin composite microcylinders within each group). Within rows, similar capital letters denote no statistically significant difference, while similar lowercase letters within columns reveal no statistically significant difference (Tukey test, p≥0.05).*

failure was found as the predominant failure mode, including partial adhesive failure at the adhesive/enamel interface and partial cohesive failure in adhesive. When it was bonded to the remineralized group with CPP-ACFP, an equal predominance for types A, B, and C failure modes was recorded. Figures 3-6 show representative ESEMs for the predominant failure modes of the tested groups.

DISCUSSION

The results of the current study revealed that the μSBS values of CPP-ACP and CPP-ACFP remineralized enamel specimens were not different from each other or from those of the control group. Thus, the first null hypothesis is accepted. For the remineralized enamel using CPP-ACP, some previous studies[12-14] also had the same findings, despite the fact that these studies used different demineralization and remineralization protocols. Regarding the CPP-ACFP group, the present study is the first of its kind to test the effect of remineralization with CPP-ACFP on bonding of self-etch adhesives to enamel; thus, no literature was available with which to compare our results. Based on the obtained results in the present study, it would be reasonable to infer that like CPP-ACP,[14] CPP-ACFP regained the enamel bonding to a comparable level compared with the sound enamel. Meanwhile, it should be

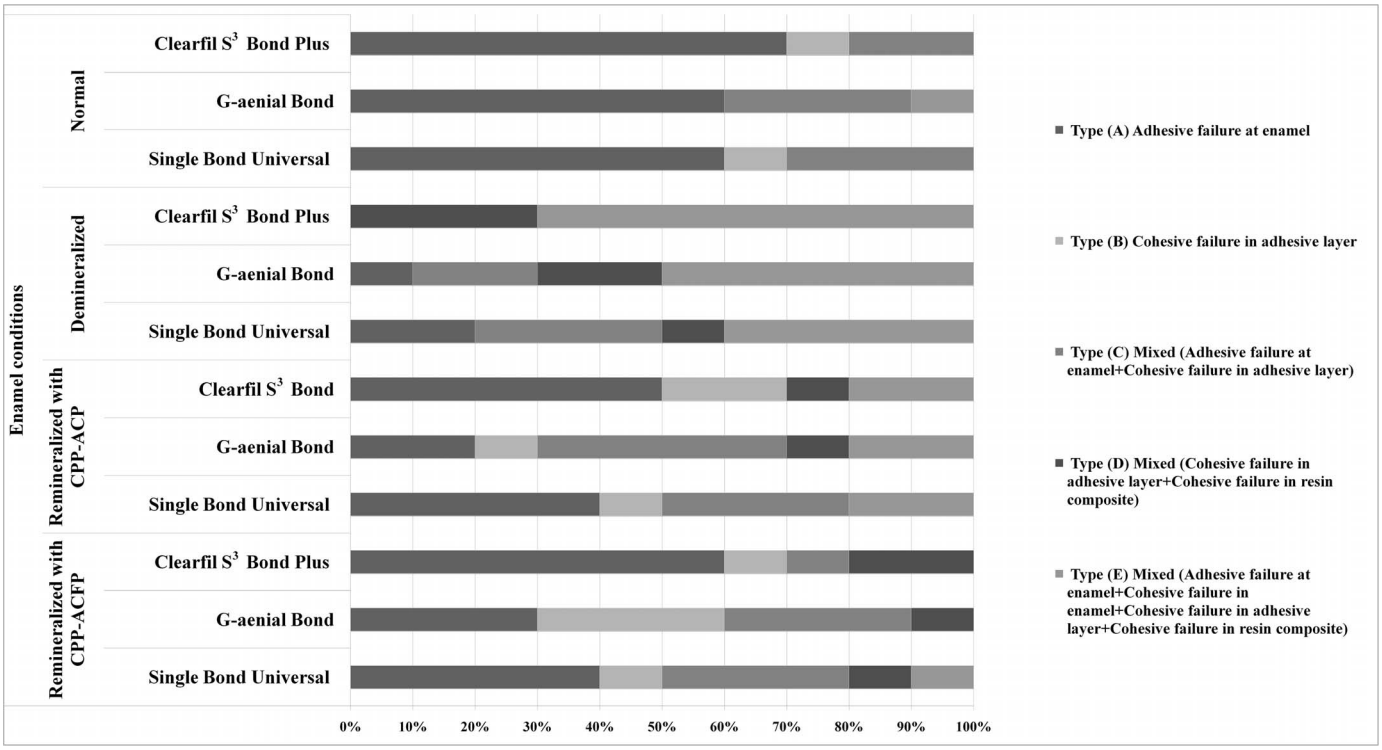

Figure 2. *The distribution of failure modes for all tested groups.*

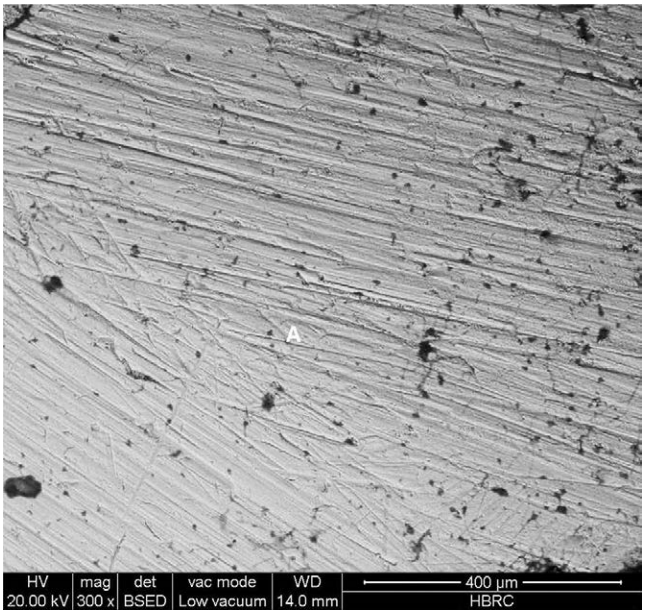

Figure 3. *ESEM photomicrograph showing adhesive failure at enamel/adhesive interface (A) representing failure mode of Clearfil S^3 Bond Plus bonded to control group.*

noted that remineralized enamel does not always have enamel prisms, and it is sometimes composed of a highly dense compaction of calcium phosphate and fluoride.[12] In addition, residual CPP-ACP complexes may remain on the enamel surface and be incorporated into the bonding layer or inhibit the bond between the adhesive system and enamel.[21]

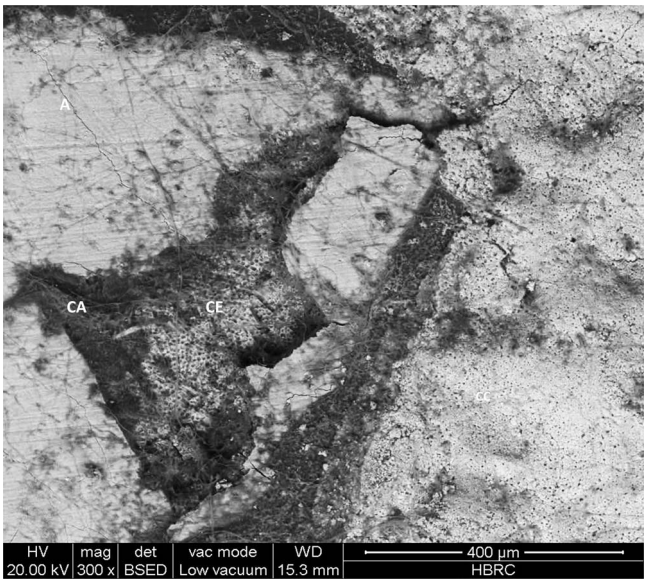

Figure 4. *ESEM photomicrograph showing mixed failure: adhesive failure at enamel/adhesive interface (A), cohesive failure in enamel (CE), and cohesive failure in resin composite (CC) and in adhesive (CA) of Single Bond Universal bonded to demineralized group.*

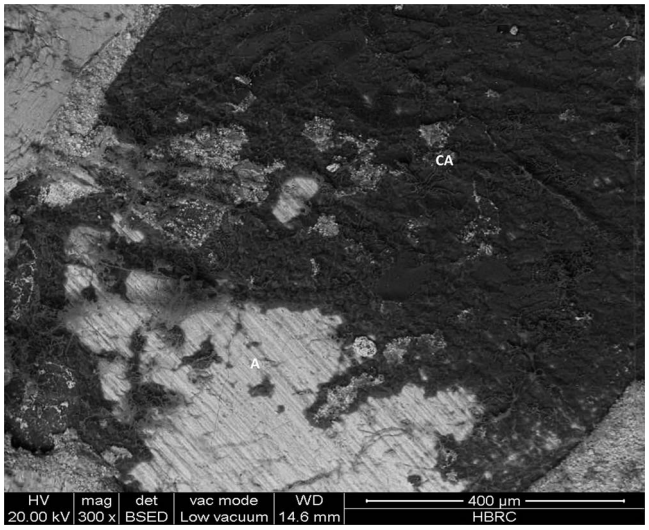

Figure 5. *ESEM photomicrograph showing mixed failure: adhesive failure at enamel/adhesive interface (A) and cohesive failure in the adhesive (CA) of G-aenial Bond bonded to remineralized group with CPP-ACP.*

In the current study, the lowest μSBS values were recorded when the adhesive systems bonded to demineralized enamel. This corroborates with previous studies.[12,14,22] This finding may be attributed to the poor quality of the enamel surface available for bonding, which hinders proper micromechanical interlock. Moreover, mixed failure with cohesive failure in enamel was more often seen in the demineralized group. This finding was in accordance

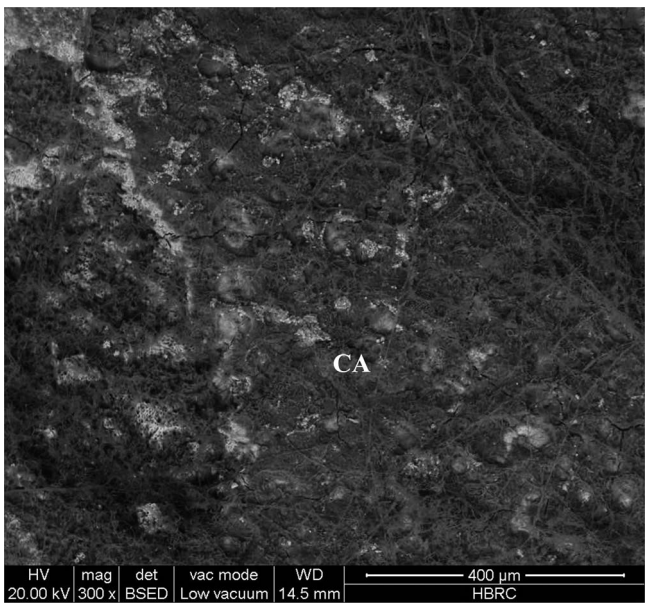

Figure 6. *ESEM photomicrograph showing cohesive failure in the adhesive (CA) of G-aenial Bond bonded to remineralized group with CPP-ACFP.*

with that of the previous study.[23] The reported cohesive failures in enamel may be due to the brittleness of demineralized enamel, which is more likely to fracture under stress. In addition, the self-etching adhesive did not penetrate so deeply as to reinforce the demineralized enamel.[23] This led us to consider demineralized enamel as an improper substrate for bonding, and it should be either remineralized prior to bonding or removed.

Three types of single-step self-etch adhesive systems were selected for this study. No variations in bond strength values among the tested adhesive systems with any of the enamel conditions were recorded. Therefore, the second null hypothesis was not rejected. These similar bond values were recorded despite the differences among them in terms of composition, pH, and type of solvent. Researchers[16,24,25] found that pH alone is not the sole parameter for achieving a good bond. Thus, it appears that other factors, such as the ability of the adhesive to form a chemical bond to enamel and the strength of the adhesive system itself, significantly influence the bond values to enamel. Previous studies[16,26-29] have reported the effective bonding of Clearfil S^3 Bond Plus adhesive system to enamel. The material is stable as a result of the presence of 10–methacryloyloxydecyl dihydrogen phosphate (10-MDP), a molecule with chemical affinity for dental tissues. The two hydroxyl groups in 10-MDP chelate the calcium of the tooth structure. Additionally, Clearfil S^3 Bond Plus adhesive system may resist mechanical fatigue, since it has been reported[27] to be more resistant to mechanical stress. With regard to the G-aenial Bond adhesive system, this 2-hydroxyethyl methacrylate–free, one-step adhesive is known to induce phase separation and therefore requires post-application 'strong' air-thinning. This strong air-thinning is very feasible on flat surfaces, leading to a quite thin and more uniform adhesive layer, which is in contrast to what actually happens in clinical conditions.[30] The Single Bond Universal adhesive system was expected to achieve superior bonding to enamel in comparison to other tested adhesives. This was thought to be due to the existence of 10-MDP and Vitrebond copolymer, with their ability to bond chemically to calcium in enamel hydroxyapatite, providing stable and durable interfaces.[31] In contradiction to our expectation, this adhesive failed to reveal better bonding. At the same time, the recoded failure modes for this adhesive were not different from those recorded with Clearfil S^3 Bond Plus adhesive system.

Nevertheless, attaining very high bond strengths is not necessarily an indicator of clinical success. It would appear that other parameters, such as chemical interaction with the tooth surface and bond stability over the long term, may be important for the clinical success of bonded restorations.[16] This encourages further research that focuses on the long-term bond durability of these adhesives to CPP-ACP and CPP-ACFP remineralized enamel.

CONCLUSIONS

All single-step self-etch adhesives revealed comparable μSBS values to ground enamel and enamel remineralized with CPP-ACP or CPP-ACFP. Bonding to demineralized enamel was ineffective. With any enamel condition, no tested single-step self-etch adhesive was superior in its bonding.

Conflict of Interest

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

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Lesion Activity Assessment (LAA) in Conjunction With International Caries Detection and Assessment System (ICDAS) for Occlusal Caries Diagnosis in Permanent Teeth

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SM Paiva • EF Ferreira • LCN Ferreira
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Clinical Relevance

The ICDAS proved to be a reproducible method with good performance in detecting carious lesions in the dentin threshold. The ICDAS-LAA criteria were reproducible to assess caries activity, but with a low degree of accuracy.

SUMMARY

Objective: The aim of this study was to investigate the clinical performance and to validate the Lesion Activity Assessment (LAA) in conjunction with the International Caries Detection and Assessment System (ICDAS) for occlusal caries diagnosis in permanent teeth.

Methods: Patients with erupted or partially erupted third molars were recruited from the surgery clinic of the School of Dentistry of the Universidade Federal de Minas Gerais, Brazil. A calibrated examiner evaluated 49 teeth using the ICDAS-LAA criteria. The histologic criterion proposed by Ekstrand and others was used

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to validate severity at the thresholds D1 (outer half of the enamel), D2 (inner half of the enamel and outer third of the dentin), and D3 (inner or middle third of the dentin). Lesion activity was validated using 0.1% methyl red solution.

Results: The method demonstrated good reliability (weighted kappa for severity=0.60; unweighted kappa for activity=0.61). The ICDAS presented a higher performance for lesion detection (area under the receiver operating characteristic curve [Az]=0.79) using the threshold D3. At the thresholds D1 and D2, the results for Az were 0.57 and 0.74, respectively. Regarding the ICDAS-LAA, Az = 0.59.

Conclusions: Clinical protocols can use ICDAS for the severity diagnosis of occlusal caries, but the LAA performance was poor.

INTRODUCTION

Epidemiologic studies have demonstrated that dental caries continues to be a public health problem worldwide. Changes in the preferential sites of occurrence and behavior of carious lesions have made diagnosis difficult. With its complex anatomy, the occlusal surface of molars favors the buildup of biofilm. Such sites are more vulnerable to the development of caries, so there is increased concern regarding the detection of these lesions[1-3] and their activity status.

The International Caries Detection and Assessment System (ICDAS) was developed to improve the accuracy of clinical decisions on caries assessment. The system was modified in 2005 by adding criteria for lesion activity assessment (LAA).[4] The LAA is based on the study of Nyvad and others[5] and was validated by Ekstrand and others.[6]

The LAA is one available system that may be used in conjunction with ICDAS to assess caries activity, although there are other options.[5,7] Assessing lesion activity is important because of the decreasing incidence of caries in contemporary populations and because the overall slow rate of caries progression requires a more sensitive diagnostic criterion. A diagnostic system that reflects the dynamic nature of caries is crucial to evaluate the effect of various nonoperative interventions for caries control.[5] Furthermore, more accurate treatment decisions can be made when one can distinguish active and non-active carious lesions in conjunction with their depths.[6]

One clinical study tested the accuracy of the scoring system for assessing caries activity (LAA) in permanent and primary teeth, using an impression material (Clinpro Cario Diagnosis, Full Arch Lactic Acid Locator, 3M ESPE, Maplewood, MN, USA) as a validation method.[6] Only one *in vivo* study has assessed the activity of carious lesions in the primary dentition, and it included the ICDAS-LAA with histologic validation (0.1% methyl red dye). The authors concluded that the ICDAS-LAA seems to overestimate the activity in the cavitated lesions.[8]

However, clinical studies have not yet been conducted to assess the reliability and accuracy of the ICDAS-LAA to evaluate occlusal caries activity in permanent teeth incorporating histologic validation. The aim of this study was to investigate the clinical performance and to validate with histologic exam the ICDAS-LAA for diagnosing occlusal caries in permanent teeth.

METHODS AND MATERIALS

Ethical Considerations

This study was approved by the ethics committee of the Universidade Federal de Minas Gerais, Brazil (ETIC: 0484.0.203.000-10). The participants received information on the objectives and procedures and signed a statement of informed consent agreeing to participate in the study.

Sample Selection

Initially, 42 volunteers (20 males and 22 females; age range 16 to 39 years) participated in the study. All subjects were scheduled for extractions at the surgery clinic of the School of Dentistry of the Universidade Federal de Minas Gerais, Brazil. Erupted or partially erupted third molars were selected based on the following inclusion criteria: an occlusal surface that was free for exam; lack of restorations or sealants on the occlusal surface; absence of extensive carious lesions on the buccal, lingual, and proximal surfaces; and absence of advanced degrees of hypoplastic and fluorotic defects. The teeth were examined visually using the ICDAS-LAA criteria by one researcher (FVMDC) before the surgical procedure. All surgical procedures were performed at the aforementioned surgical clinic.

Calibration Process

One examiner (FVMDC) underwent a calibration process according to the ICDAS Coordinating Committee *Criteria Manual*.[9] The benchmark examiner (SMP) presented the criteria in a 90-minute class. Then, the researcher and the benchmark examiner evaluated 36 tooth images and discussed the criteria

and codes used to assess the lesion severity and activity. One week after the theoretical calibration, clinical training was carried out on third molars (n=20). The teeth were cleaned with a pumice and water paste using a brush, followed by rinsing. First, 10 teeth were examined under a dental operating light with isolation by cotton rolls, using a 3-in-1 syringe, a plane dental mirror, and a World Health Organization (WHO) probe (Hu-Friedy, Rio de Janeiro, Brazil). During the visual exam, the benchmark examiner and the researcher discussed the ICDAS-LAA criteria and codes. After the clinical training, they independently examined the other 10 teeth and recorded the codes. One week later, they repeated the exam to determine intra- and inter-examiner agreement. The weighted kappa index for interexaminer agreement was 0.67 for severity, and the simple kappa index was 0.70 for activity. The weighted kappa index for intraexaminer agreement was 0.84 for severity, and the simple kappa index was 1.0 for activity.[10]

Visual Clinical Exam

The clinical exams were conducted at the dental offices following the ICDAS Coordinating Committee *Criteria Manual* recommendations.[9] The teeth were examined after cleaning with a brush and a pumice and water paste, followed by rinsing. The clinical exam was performed under a dental operating light with isolation by cotton rolls, using a 3-in-1 syringe, a plane dental mirror and a WHO probe. The examiner chose the site presenting the severest lesion and recorded on a schematic drawing of the tooth occlusal surface. Then, the code was assigned to the site as follows: 0 = no or slight change in enamel translucency after prolonged air drying for 5 seconds; 1 = first visual change in enamel (seen only after prolonged air drying or restricted to within the confines of a pit or fissure); 2 = distinct visual changes in enamel; 3 = localized enamel breakdown in opaque or discolored enamel (without visual signs of dentinal involvement); 4 = underlying dark shadow from dentin; 5 = distinct cavity with visible dentin; and 6 = extensive distinct cavity with visible dentin (involving more than half of the surface).

Based on the ICDAS-LAA criteria, activity was defined using the combined knowledge obtained from the visual appearance, tactile feel, and potential for biofilm accumulation. All sites were considered natural biofilm stagnation area as the teeth were not in occlusion. The appearance and texture criteria were verified with a WHO probe without assigning scores. The ICDAS codes 1, 2, and 3 were considered active lesions when the enamel surface was whitish/yellowish opaque, showed loss of luster, and felt rough when the tip of the probe was moved gently across the surface, which would indicate that the enamel was rough because of caries and not because of staining or partly mineralized debris, calculus, or anatomy. Inactive enamel lesions were recorded when the surface was whitish, brownish, or black and the enamel was shiny and felt hard and smooth when the tip of the probe was moved gently across the surface. When the enamel was smooth to probing, superficial defects were accepted if they were open and the borders were smooth to probing. [9] An ICDAS code 4 was considered active. ICDAS codes 5 and 6 were considered active when the dentin surface felt soft or leathery on gently probing. An inactive lesion was recorded when the dentin was shiny and felt hard on gentle probing.[6]

After 7 days, the exam was repeated in 10 randomly selected teeth to calculate intraexaminer reproducibility.

Histologic Validation

Immediately after extraction, the teeth were scraped to remove debris, and stored at −20°C for up to 30 days.[7] Then they were thawed and sectioned perpendicularly to the carious site using a diamond blade (series 15 LC NO. 11-4276, Buhler Ltd, Lake Bluff, IL, USA) mounted on a precision saw (Isomet, Buhler Ltd). One trained examiner (k=0.77) blinded to the visual examination assessed the sections under a stereomicroscope (Carl Zeiss, Oberkochen, Germany) at 32× magnification. Carious lesions were defined based on the extent of a whitish or brownish demineralization zone in the occlusal-pulp direction and classified based on the following criteria: 0 = no demineralization, 1 = demineralization limited to the outer half of the enamel thickness, 2 = demineralization between the inner half of the enamel and the outer third of the dentin, 3 = demineralization in the middle third of the dentin, and 4 = demineralization in the inner third of the dentin.[11]

Validation of Caries Activity

One drop of 0.1% methyl red aqueous solution (Merck, Rio de Janeiro, Brazil) buffered with sodium hydroxide (pH=7) was applied to the histologic slices. After 1 minute, the excess solution was removed with absorbent paper, and the slices were examined under a stereomicroscope at 32× magnification. The staining on the enamel and dentin was recorded as yellow (indicative of inactive lesion: pH>5.5) or red (indicative of active lesion: pH<5.5).[11]

Table 1: *Comparison of ICDAS-LAA Activity Criteria and Methyl Red Stain (Gold Standard)*

ICDAS-LAA All Surfaces*	Methyl Red Stain		
	Active	Inactive	Total
Active	11	21	32
Inactive	3	14	17
Total	14	35	49
Enamel**			
Active	7	14	21
Inactive	2	10	12
Total	9	24	33
Dentin***			
Active	4	2	6
Inactive	1	0	1
Total	5	2	7

[a] *Scores 0–4 according to the histologic criteria.*
[b] *Scores 1–2 according to the histologic criteria.*
[c] *Scores 3–4 according to the histologic criteria.*

Statistical Analysis

Intraexaminer agreement for the ICDAS severity and activity criteria was determined using the weighted and simple Kappa statistic, respectively. The validity of the diagnostic tests was expressed as sensitivity, specificity, accuracy, and 95% confidence intervals (CIs) at the different thresholds (D1, D2, and D3). The cutoff points for ICDAS were defined as D1 (0 healthy and 1 to 6 carious), D2 (0 and 1 healthy and 2 to 6 carious), and D3 (0, 1, and 2 healthy and 3 to 6 carious). The histologic exam results were classified as D1 (0 healthy and 1 to 4 carious), D2 (0 and 1 healthy and 2 to 4 carious), and D3 (0, 1, and 2 healthy and 3 to 4 carious). Analysis of the area under the receiver operating characteristic curve (Az) and 95% CI was used to evaluate the performance of the diagnostic systems. Spearman correlation coefficients were calculated to determine correlations between the histologic exam and the ICDAS. Caries activity was analyzed after the data were dichotomized into healthy sites and inactive lesions or active lesions. Statistical analysis was also performed using only one tooth per patient, which was selected by computer randomization. The SPSS 14 program for Windows (Chicago, IL, USA) was used for statistical analysis.

RESULTS

Sample Characteristics

Initially, 42 people agreed to participate in the study. After the teeth were extracted, 33 subjects remained (19 females, 14 males; age range, 16 to 39 years old). All participants reported the use of a 0.7 mg/l F water supply and fluoride toothpaste.

Table 2: *Comparison of the Histologic Gold Standard With ICDAS Severity Criteria Cut-off Points*

ICDAS	Gold Standard					
	0	1	2	3	4	Total
0	4	7	5	0	0	16
1	1	2	3	2	0	8
2	3	1	10	0	0	14
3	1	0	3	1	0	5
4	0	0	1	0	1	2
5	0	0	1	1	1	3
6	0	0	0	0	1	1
Total	9	10	23	4	3	49

Abbreviation: ICDAS, International Caries Detection and Assessment System Lesion Activity Assessment.

The sample consisted of 49 sites obtained from 49 extracted teeth: 11 right maxillary third molars, 10 left maxillary third molars, 15 right lower third molars, and 13 left lower third molars. The losses were due to teeth sectioned during surgery. There was no loss in teeth sectioned for microscopic examination.

Diagnosis of caries activity using methyl red showed 14 (28.5%) active and 35 (71.4%) inactive lesions. Methyl red identified 9 active enamel lesions and 5 active lesions in dentin. The LAA correctly classified 11 lesions (7 enamel and 4 dentin) (Table 1).

Histologic examination showed nine healthy sites (16.32%), 10 (20.4%) lesions extending to the outer half of the enamel (D1), 23 (46.9%) lesions extending to the inner half of the enamel and outer third of the dentin (D2), and 7 (14.28%) lesions to the inner or middle third of the dentin (D3) (Table 2). No significant differences were found between the analysis performed with one tooth per patient and that performed with more than one tooth per patient.

ICDAS-LAA Activity Criteria

The intraexaminer agreement for the ICDAS-LAA activity criteria was $k = 0.61$, which was considered good.[12] The ICDAS-LAA diagnosed 32 active lesions (65.1%), 16 (32.6%) sound sites, and one (2.0%) inactive lesion. Sound sites and inactive lesions were grouped for analysis in histologic and clinical outcomes. Compared with the methyl red gold standard, 25 sites (51.0%) were correctly classified (Table 1). Of the seven dentin lesions, LAA classified six as active and one as inactive lesions.

Table 3: *Specificity, Sensitivity, Accuracy, and Az (95% CI) for ICDAS-LAA in Relation to Methyl Red Stain*

ICDAS-LAA	Specificity	Sensitivity	Accuracy	Az
All surfaces[a]	0.40 (0.24–0.58)	0.78 (0.49–0.95)	0.51 (0.37–0.63)	0.59 (0.42–0.77)
Enamel[b]	0.42 (0.22–0.63)	0.78 (0.40–0.97)	0.51 (0.34–0.68)	0.59 (0.38–0.81)
Dentin[c]	0	0.80 (0.28–0.99)	0 (0–0.84)	0.40 (−0.064–0.86)

Abbreviations: Az, area under the receiver operating characteristic curve; CI, confidence interval; ICDAS-LAA Lesion Activity Assessment used in conjunction with the International Caries Detection and Assessment System.
[a] Scores 0–4 according to the histologic criteria.
[b] Scores 1–2 according to the histologic criteria.
[c] Scores 3–4 according to the histologic criteria.

The sensitivity, specificity, accuracy and Az for the ICDAS-LAA activity criteria were 0.78, 0.40, 0.51 and 0.59, respectively (Table 3).

ICDAS-LAA Severity Criteria

The intraexaminer agreement for the ICDAS-LAA severity criteria was kw = 0.60, which was considered good. [12] The disagreements were related to noncavitated lesions scored 0 to 2 (Table 2).

The ICDAS determined 67.3% of cavitated and noncavitated lesions (44.8% enamel and 22.4% dentin lesions). In relation to the gold standard, correct diagnoses were obtained for four healthy sites (8.16%), two D1 (4.0%), ten D2 (20.4%), and five D3 (10.2%) lesions (Table 2). The Spearman correlation coefficient (0.515) demonstrated a moderate correlation with the gold standard (p=0.01). Table 4 displays the specificity, sensitivity, accuracy, and Az for the ICDAS severity criteria. The highest sensitivity (0.71), specificity (0.86), accuracy (0.83), and Az (0.79) values were observed at the D3 threshold.

DISCUSSION

This study investigated the clinical performance and validated the LAA used in conjunction with the ICDAS for the diagnosis of occlusal caries in permanent teeth. Assessment of caries activity using the ICDAS-LAA criteria was reproducible but had low accuracy.

Sensitivity and specificity are test properties used to quantify diagnostic ability. Sensitivity is the proportion of true positives that are correctly identified by the test. Specificity is the proportion of true negatives that are correctly identified by the test. Sensitivity and specificity are proportions; thus, CIs were calculated to express their variabilities.[13]

The presence of dental biofilm is a predictor of lesion activity, but the ICDAS system requires that it be removed before the initial examination to accurately access the lesion. Thus, biofilm could not be used. Therefore, the criteria of whether or not the lesion was located in a stagnation area was used as a substitute for biofilm accumulation as, under normal conditions, lesion progression will occur only in biofilm stagnation areas.[6] In the present study, the teeth were not in occlusion and all the occlusal surfaces were considered stagnation areas. This was a shortcoming of the study as all the sites were considered a stagnation area without actually assessing the presence of biofilm.

The low accuracy found for LAA in the present study may be because of the difficulty of examining the occlusal surface of third molars. Their distal position limits the light access, and gingival flaps hamper the maintenance of dry surfaces for the visual examination. Furthermore, the use of methyl red for the activity validation can be considered a subjective method that has limitations.[8] Nowadays, no accepted gold standard is available to differentiate between an active and an arrested lesion upon single examination. To overcome this lack of an accepted gold standard, a known theoretical condition, which is associated with caries activity, can be

Table 4: *Specificity, Sensitivity, Accuracy, and Az (95% CI) for ICDAS at the D1, D2, and D3 Thresholds*

ICDAS	D1	D2	D3
Specificity	0.44 (0.30–0.57)	0.68 (0.55–0.81)	0.86 (0.76–0.94)
Sensitivity	0.71 (0.59–0.83)	0.66 (0.53–0.79)	0.71 (0.59–0.83)
Accuracy	0.65 (0.52–0.78)	0.67 (0.54–0.80)	0.83 (0.73–0.93)
Az	0.57 (0.44–0.70)	0.74 (0.61–0.86)	0.79 (0.67–0.90)

Abbreviations: Az, area under the receiver operating characteristic curve; CI, confidence interval; ICDAS International Caries Detection and Assessment System; D1, outer half of the enamel; D2, the inner half of the enamel and outer third of the dentin; D3, the inner or middle third of the dentin.

used as "construct validity."[6] The critical pH for the demineralization of enamel is 5.5,[7,11] and methyl red changes from yellow to red in the pH range of 4.4 to 6.0.[11] Based on this pH range, methyl red may not be capable of adequately classifying some lesions. Certain active lesions with only a slight degree of demineralization may exhibit yellowish staining.[8,11]

A previous study compared methyl red dye to polarized light microscopy examination of teeth sections imbibed in quinolone to assess caries activity based on acid production. Comparison of these techniques showed 85% agreement. Considering the tooth loss after sectioning for polarized light microscopy, the use of the simple dye method was justified to histologically distinguish active and inactive lesions.[11] Therefore, this method was chosen because of practicality and comparability with other *in vivo* studies. [8,11]

Intraexaminer agreement for the activity criterion was lower than that reported in clinical studies on primary[8] and permanent teeth.[6] This could be due to the subjectivity of the criterion as well as the different experiences of the examiners using ICDAS-LAA system.

The gold standard showed 81.6% of carious lesions involving enamel or dentin. Similar results were found for third molars examined in 19- to 30-year-old Brazilians (81.8%).[14] A frequency of 95% was reported in premolars and molars in 18- to 35-year-old Brazilians after histologic validation.[15] These high caries frequencies may be attributed to the patients' risk of lesion development during the eruption of permanent molars. The complex occlusal anatomy, the difficulty in performing hygiene, and the lack of occlusal contact can contribute to the stagnation of biofilm.[11,16,17] The use of third molars is a possible shortcoming of validation studies in permanent teeth. The present study could not be carried out on first and second molars because they are rarely extracted. First and second permanent molars have a more pronounced groove-fossa system than third molars.[11] However, third molars may be difficult to clean, simulating the condition in which patients are not removing biofilm and occlusal caries can develop within a year after tooth eruption.[17]

A risk assessment for dental caries was not performed and that could have affected the outcome. If we had selected only high- or low-risk patients the caries spectrum could have been different, thereby influencing test performance. However, we focused on selecting patients with teeth available for histologic validation. Risk assessment would be crucial if we aimed to associate the dental caries with the patient's related variables, but it was not the purpose of this study.

This *in vivo* study demonstrates that the ICDAS performance was higher on detecting dentin lesions than enamel lesions. The accuracy for detecting early demineralization on enamel was moderate. The Az showed that ICDAS was poor regarding the diagnosis of dental caries at the D1 and acceptable at the D2 and D3 thresholds.

We expected higher performance of ICDAS for identifying enamel lesions at different depths as Az = 0.86 were previously found for permanent[14] and primary teeth[8] at the threshold D1. The differences in caries frequencies diagnosed using the gold standard and ICDAS may be attributed to the examiner's difficulty in visually differentiating healthy surfaces from those with early signs of enamel caries (ICDAS code 1). A great proportion of false-negatives were found in the present study. From the 16 healthy sites classified by the ICDAS, only 4 were confirmed by the gold standard. Furthermore, from the nine healthy surfaces identified by the gold standard, ICDAS identified five as carious lesions. Healthy sites may be classified as carious, likely because of areas of discoloration, fluorosis, or developmental defects. At the D1 threshold, false-positive rates have greater impact than false-negatives on treatment decision because implementing operative procedures at a healthy site would be the worst decision.

The Az at the threshold D3 was similar to that found in another validation study on permanent teeth.[14] However, in the present study, the frequency of deeper dentin lesions was 14.3%. The sample presented few obviously cavitated lesions and affected the performance of the method, as such lesions are easier to detect.

Detection of dental caries in the early stages is more prone to disagreement between examiners.[8] The visual exam may be impaired by difficulties in examining third molars because of their location and the patient's discomfort during longer exams.[18] Despite these aspects, the intraexaminer agreement regarding ICDAS severity was 0.61 in the present study, similar to that described in *in vivo* studies[19,20] with permanent molars. The exclusion of code 1 of the ICDAS from the analyses, which customarily generates greater disagreement, may explain the higher agreement values reported in a Colombian study.[21]

The correlation between the ICDAS and histologic exam was moderate and lower than that reported in a clinical study with primary teeth.[8] However, another *in vivo* study found lower correlation in permanent teeth.[14] A correlation coefficient of 0.7 or more would indicate a good correlation between the two methods.[14]

The variables evaluated in multiple teeth from one subject are not independent as the individual characteristics play a crucial role in caries development. Nevertheless, the ICDAS-LAA performance was similar when the unit of analysis was one subject or one tooth, resembling findings of previous studies. [6,8]

This study selected a convenience sample that could have included bias. However, the sample size was similar to that of other studies.[8,15] A power of 80% was found using type I error equal to 0.05 and a difference of sensitivities equal to 0.23 (0.93 from Diniz and others[14] compared with 0.70 from our study).[22]

The advent of systematic methods offers dentists the opportunity to enhance their diagnostic skills. However, the proper decision regarding the presence/absence of caries as well as the severity and likely activity of this condition, combined with sociobehavioral aspects of the patient, remains the responsibility of the dentist.

CONCLUSION

The ICDAS proved to be a reproducible method with good performance in detecting carious lesions in the dentin. The ICDAS-LAA criteria were reproducible to assess caries activity but had low accuracy.

Conflict of Interest

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

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Evaluation of the Radiopacities of Bulk-fill Restoratives Using Two Digital Radiography Systems

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Clinical Relevance

Bulk-fill restoratives had higher radiopacity values than dentin and enamel at varying thicknesses, which makes these restoratives suitable for radiographic visualization of caries.

SUMMARY

This study investigated the radiopacity values of bulk-fill restoratives by using two digital radiography systems. Nine bulk-fill restoratives and a conventional composite were used in the study. Six disc-shaped specimens were prepared from each of these materials, three each at thicknesses of 1 mm and 2 mm, and tooth slices with these same thicknesses were obtained. As a control, an aluminum step wedge varying in thickness from 0.5 to 10 mm in was used. Three specimens of each of the materials, together with the tooth slice and the aluminum step wedge, were placed over a complementary metal oxide semiconductor (CMOS) sensor and a storage photostimulable phosphor (PPS) plate system and exposed using a dental x-ray unit. The images were analyzed using a software program to measure the mean gray values (MGVs). Five measurements were obtained from each of the restorative materials, the enamel, the dentin, and the stepwedge. The MGVs were converted to the equivalent aluminum thicknesses. Three-way analysis of variance (ANOVA) was used to determine the significance of the differences among the groups. A Tukey test was applied for pairwise comparisons ($p<0.05$). All composite-based restoratives were found to have greater radiopacities than enamel or dentin. Equia Fil had the lowest radiopacity value. Radiopacity increased as the thicknesses of the restorative material increased. The CMOS system showed significantly higher radiopacity values than the

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PSP system. In conclusion, all investigated bulk-fill restoratives passed the International Organization for Standardization and American National Standard Institute/American Dental Association requirements for radiopacity values when evaluated with the two digital radiography systems.

INTRODUCTION

Radiopacity is an essential property for all restorative materials[1] and one of the revised five requirements that a dental material must meet according to the American Dental Association (ADA), Council on Dental Materials, Instruments and Equipment.[2] A material with adequate radiopacity allows detection of secondary caries and distinguishes the caries from the restorative material and surrounding tooth structure. In addition, the proximity of the pulp, marginal defects, overhangs, and open margins can be easily seen.[3,4] Both the International Organization for Standardization (ISO) and American National Standard Institute (ANSI)/ADA have recommended standardized procedures for quantifying material radiopacity using aluminum as a reference.[5] According to the last declaration in IS0 4049:2009, if the manufacturer claims that a material is radiopaque, the radiopacity should be equal to or greater than that of the same thickness of aluminum and no less than 0.5 mm below any value claimed by the manufacturer.[6]

Several factors may affect the radiopacity of dental materials, including the type of restoration, the processing system (digital or conventional), the type of digital sensors, the device setup parameters (exposure time, voltage, and target distance), and material thickness and composition.[7]

Since 1989, digital systems have been used in dental practice and provide numerous advantages over conventional radiographic systems. These benefits include shorter radiation exposure for operator and patient, faster and easier operation, a convenient method to store image and exchange data for referrals, and elimination of the need for film development chemicals.[8] Although traditional film development may produce significant variations in the final radiograph, digital systems provide more consistent results.[5,9,10] However, depending on the radiographic system used, image-modifying procedures, and location on the dental arch, dental materials can show significant differences in radiopacity when measured using digital versus conventional systems.[11,12] In laboratory research, digital systems also offer advantages in evaluating the radiopacity of dental materials. By using the image software programs of these systems, the mean gray values (MGVs) of each material or structure on the radiograph can be calculated within a scale ranging between 0 (black) and 255 (white).[3]

Several types of sensors are used in digital radiographic systems: charge-coupled devices (CCDs); complementary metal oxide semiconductor (CMOS), also referred to as wired sensors or direct systems; and photostimulable phosphor (PSP) plates, also referred to as wireless sensor or indirect systems. Both direct and indirect digital radiograph systems allow quantitative measurements, enlargement to focus on areas of interest, color correction, and adjustment of contrast and density to sharpen and improve image quality. However, the exposure times required by direct systems are lower than those for indirect systems, and the image quality is higher.[11] Although CCD and CMOS use basically the same approach, it has been reported that CMOS sensor values were comparable to those of the CCD sensors but require higher exposure times.[13]

Although the radiopacity of dental materials may be affected by several factors, composition of the materials seems to be the most important. With improvements in the chemical compositions of resin-based composites and the variety of filler reinforcements in the material compositions, many categories of dental materials are now available. As long as new materials are released to the market, ongoing studies to evaluate the radiopacity of dental materials are important to avoid misinterpretation during image diagnosis.[14] Bulk-fill restoratives were introduced as a new category of low- and high-viscosity composites for Class I and Class II restorations. Instead of using the current incremental placement technique, this new material can be placed in a 4-mm thickness because of its particular qualities compared with restoratives with similar properties.[15] It is assumed that the composition of bulk-fill restoratives does not differ markedly from that of current incrementally filled conventional resin composites. However, the differing chemistry of the monomeric resin formulations and filler characteristics (type, volume fraction, density, and particle size and distribution) of bulk-fill restoratives may affect radiologic characteristics, as did the depth of cure and mechanical properties in the study by Finan and others.[16] However, there are no comparative data regarding the radiopacity of bulk-fill restoratives. Therefore, the aim of the present study was to evaluate the radiopacities of nine recently produced bulk-fill restoratives at different

Table 1: *Materials used in the study*

Material	Radiopaque Filler Content and Filler % (wt/vol)	Manufacturer	Batch No
X-tra base	Not applicable (75/58)	Voco GmbH, Cuxhaven, Germany	1147278
Tetric N-Ceram Bulk Fill	Barium glass, ytterbium trifluoride, mixed oxide; (77/55)	Ivoclar Vivadent AG, Schaan, Liechtenstein	R72543
Tetric EvoCeram Bulk Fill	Barium aluminium silicate glass, prepolymer filler, ytterbium fluoride, and spherical mixed oxide (80/61)	Ivoclar Vivadent AG, Schaan, Liechtenstein	R82389
SonicFill	Glass, oxide, chemicals, silicon dioxide (not applicable/83)	Kerr Corporation, Orange, CA, USA	3851730
X-tra fill	Barium aluminium silicate glass (86/70)	Voco GmbH, Cuxhaven, Germany	1245232
SDR Bulk Fill	Barium alumino fluoro borosilicate glass, strontium alumino fluoro silicate glass (68/44)	Dentsply DeTrey, Konstanz, Germany	1001086
Quixfil	Zirconium oxide, silicon dioxide (86/66)	Dentsply DeTrey, Konstanz, Germany	121000
Equia Fil	Fluoro alumino silicate glass (not applicable)	GC Corp, Tokyo, Japan	1203121
Filtek Bulk Fill	Zirconia/silica, ytterbium trifluoride (64/42)	3M ESPE, St Paul, MN, USA	N435626
Clearfil Majesty Posterior	Silanated glass ceramics, Surface-treated alumina microfiller, silanated silica filler (92/82)	Kuraray, Medical Co., Tokyo, Japan	00152

Abbreviations: CMOS, complementary metal oxide semiconductor; PSP, photostimulable phosphor.

thicknesses using two different digital radiography systems. The null hypothesis was that there was no statistically significant difference in the radiopacities of bulk-fill restoratives at different thicknesses.

METHODS AND MATERIALS

Specimen Preparation

Nine bulk-fill restoratives and a conventional composite were used in this study. Table 1 lists the materials, chemical compositions, manufacturers, and batch numbers.

The sample size was calculated considering 80% power and a significance level of 0.05 using data (effect size=4.08) obtained from the study by Lachowski and others.[3] Although according to the data from that study, 12 specimens are sufficient for analysis, a worst-case scenario was proposed with a 0.99 effect size for the current study. According to the worst-case scenario, a total sample size of 27 (n=3) was calculated considering 87% power at a significance level of 0.05. Plastic ring molds with an internal diameter measuring 6 mm and depths of 1 mm and 2 mm were used to prepare standardized specimens. Three specimens were prepared from each of the materials at each height in accordance with the manufacturers' instructions. The mold was placed on a glass microscope slide, and the materials were inserted into the mold until it was overfilled. A Mylar matrix strip was then placed on the top. A second glass slide was positioned over the strip to flatten the surfaces before curing using a light-activating source (Valo, Ultradent Products Inc, South Jordan, UT, USA). The specimens of each material were cured through the Mylar strip and glass slide. After the specimens were removed from the mold, the thicknesses were verified with a digital caliper to ensure standardization. The specimens were placed in 37°C distilled water for one day to complete the polymerization process, then maintained in moist conditions pending the radiographic procedures.

One freshly extracted human molar was used to obtain enamel and dentin specimens. The tooth was prepared by longitudinal sectioning using a slow-speed diamond saw (Isomet1000, Buehler, Lake Bluff, IL, USA), and slices measuring 1 mm and 2 mm in thickness were obtained. The tooth specimens were stored in distilled water pending evaluation.

Digital Radiography

A 99% pure aluminum step wedge with 20 incremental steps measuring 0.5 mm was used. Three specimens of each material, together with the aluminum stepwedge and a tooth specimen, were positioned over the CMOS sensor (Digora Toto, Soredex, Milwaukee, WI, USA), and the storage phosphor plate system (VistaScan, Dürr Dental, Bietigheim-Bissingen, Germany) on each of the radiographs. All specimens were placed at a distance

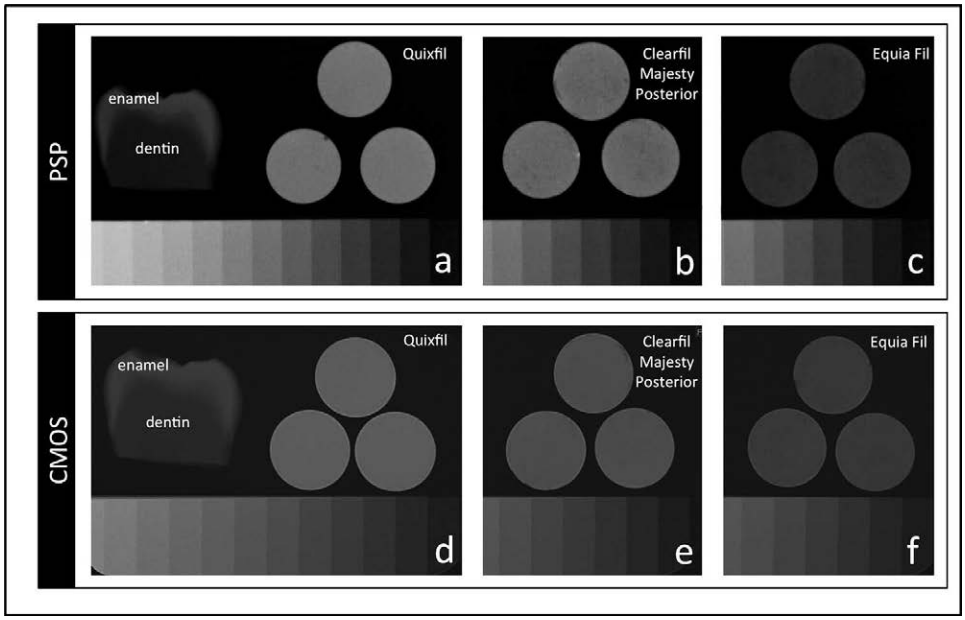

Figure 1. *Radiographic images of the enamel, dentin, aluminum step wedge in which the highest and the lowest radiopacity bulk-fill restoratives were tested in comparison to the conventional composite at thicknesses of 1 mm over the storage phosphor plate (upper) and the complementary metal oxide semiconductor sensor (lower). (a and d): Quixfil; (b and e): Clearfil Majesty Posterior; (c and f): Equia Fil.*

of 30 cm for 0.32 seconds in a dental x-ray unit (65 kVp/7 mA, Myray, Cefla Dental Group, Imola, Italy). Figures 1 and 2 show radiographic images of the enamel, dentin, aluminum step wedge, and a material at different thicknesses over the CMOS sensor and the storage phosphor plate.

The MGVs of each of the materials and tooth slices were measured on the digital radiographs using the Adobe Photoshop CS3 Extended computer program, version 10.0 (Adobe Systems, San Jose, CA, USA), in five different regions each with a 10×10 pixel area to reduce measurement bias. Selected regions avoided areas containing air bubbles or other anomalies, and measurements were taken by one evaluator, who was blinded to the identities of the materials. After the MGVs of visible steps of the aluminum step wedge on the image were calculated, a regression curve equation ($y = 10.209x + 13.265$; $R^2=0.99876$) was defined for the MGVs of further steps that could not be seen on the image because of limited dimensions of CMOS and PSP. The MGVs of each of the materials and tooth slices were then converted

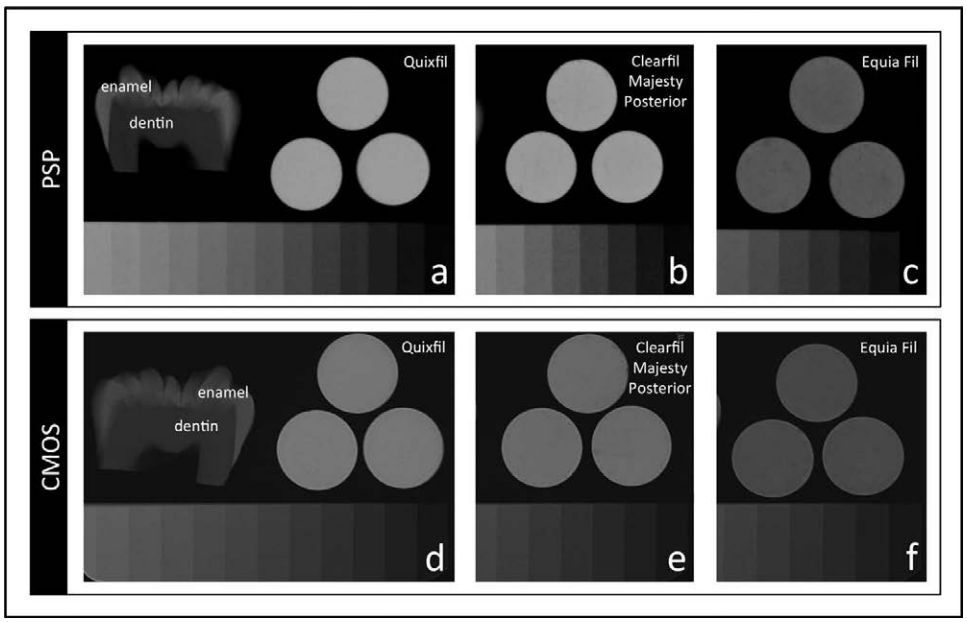

Figure 2. *Radiographic images of the enamel, dentin, aluminum step-wedge, and tested bulk-fill restoratives in comparison to the conventional composite at thicknesses of 2 mm over the storage phosphor plate (upper) and the complementary metal oxide semiconductor sensor (lower). (a and d): Quixfil. (b and e): Clearfil Majesty Posterior; (c and f): Equia Fil.*

Table 2: *Mean radiopacity values and standard deviation of the materials, enamel, and dentin at 1-mm, and 2-mm*

Material	Radiography Method	1 mm	2 mm
X-tra base	CMOS	3.38±0.09	7.06±0.04
	PSP plate	2.61±0.10	6.02±0.05
Tetric N-Ceram Bulk Fill	CMOS	3.68±0.05	7.68±0.13
	PSP plate	3.25±0.10	7.02±0.01
Tetric EvoCeram Bulk Fill	CMOS	3.67±0.07	7.73±0.02
	PSP plate	3.19±0.05	6.36±0.01
SonicFill	CMOS	3.07±0.01	6.59±0.04
	PSP plate	2.51±0.01	5.27±0.03
X-tra fill	CMOS	3.83±0.02	8.15±0.03
	PSP plate	2.93±0.07	6.59±0.01
SDR Bulk Fill	CMOS	2.92±0.09	6.27±0.08
	PSP plate	2.34±0.03	5.85±0.03
Quixfil	CMOS	3.98±0.04	7.69±0.04
	PSP plate	3.55±0.03	6.85±0.02
Clearfil Majesty Posterior	CMOS	2.98±0.08	6.44±0.05
	PSP plate	2.47±0.03	5.52±0.08
Equia Fil	CMOS	2.03±0.03	3.97±0.08
	PSP plate	1.60±0.01	3.63±0.01
Filtek Bulk Fill	CMOS	2.48±0.01	5.08±0.03
	PSP plate	2.20±0.01	4.87±0.05
Enamel	CMOS	1.99±0.04	3.63±0.03
	PSP plate	1.94±0.09	3.43±0.05
Dentin	CMOS	1.04±0.01	1.92±0.01
	PSP plate	0.94±0.23	1.83±0.05

into millimeters of aluminum (mm Al) using the following equation described by Lachkowski and others:[3]

$$\frac{A \times 0.5}{B} + \text{mm Al below material's MGV}$$

where:

A: MGV of the material – the MGV of the aluminum step wedge increment immediately below the material's MGV.
B: MGV of the aluminum step wedge increment immediately above the material's MGV – MGV of the aluminum stepwedge increment immediately below the material's MGV.
0.5: increment thickness of the aluminum stepwedge.

Statistical Analysis

All statistical analyses were performed using SPSS Statistics, version 20.0 (SPSS Inc, Chicago, IL, USA) at a significance level of 0.05 and a confidence interval of 95%. The resulting data were statistically analyzed using a three-way analysis of variance (ANOVA), considering three factors (restorative material type, thickness of material, and radiographic system). A Tukey post hoc test was used for multiple comparisons.

RESULTS

The three-way ANOVA of the radiopacity data revealed that radiopacity was significantly affected by the restorative material type, thickness of the material, and type of radiographic system used ($p<0.001$). All interactions between the evaluated factors were significant ($p<0.001$).

The mean radiopacity values and standard deviations of the enamel, dentin, and materials are shown in Table 2 and Figure 3. There was a large variation between the radiopacities of the bulk-fill restoratives. Using the CMOS system, values ranged from 2.03 to 3.98 mm Al at 1 mm and from 3.97 to 8.15 mm Al at 2 mm, Using the PSP system, values ranged from 1.60 to 3.55 mm Al at 1 mm and from 3.63 to 7.02 mm Al at 2 mm. The highest radiopacity was observed in Quixfil using both radiographic systems at 1-mm thickness (Figures 1a and 1d), X-tra Fil using CMOS at 2 mm thickness, and Tetric N-Ceram Bulk Fill using PSP at 2-mm thickness. Equia Fil had the lowest radiopacity at all parameters (Figures 1c, 1f, and 2c, 2f).

Each of the bulk-fill restoratives, except for Equia Fil and the conventional composite, showed higher radiopacities than did the dentin and enamel at all thicknesses and using both radiographic systems ($p<0.001$). Although Equia Fil had a higher radiopacity than dentin parameters ($p<0.001$), its radiopacity was similar to that of enamel (Figure 3).

When the radiopacities of the bulk-fill restoratives were compared with that of conventional composite using the CMOS, there was a significant difference between the materials, except for Sonic Fill and SDR Bulk Fill at both thicknesses ($p<0.001$) (Figures 3a and 3c). Using the PSP system, the radiopacity of the conventional composite was not significantly different from that of X-tra base, Sonic Fill, SDR Bulk Fill, or Filtek Bulk Fill at 1 mm ($p=0.086$) (Figure 3b), whereas it was significantly different at 2 mm ($p<0.001$) (Figure 3d).

The results showed that increased thicknesses in the materials that were studied correlated with significant increases in their radiopacity ($p<0.001$) (Figures 1 and 2). The CMOS system showed

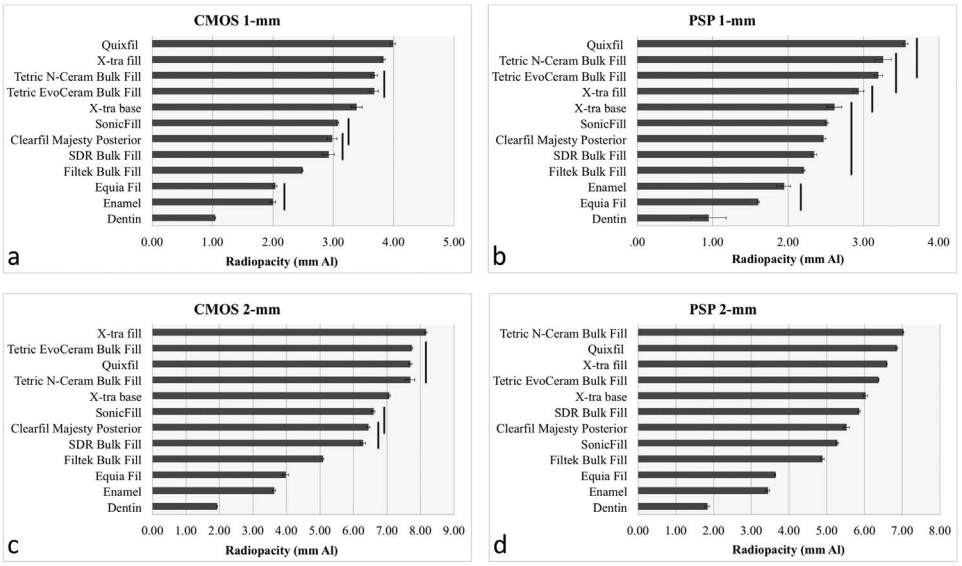

Figure 3. *The mean values of radiopacity (millimeters of aluminum) and standard deviations of nine bulk-fill restoratives and the conventional composite in comparison to enamel and dentin. (a): 1-mm thickness on complementary metal oxide semiconductor (CMOS); (b): 1-mm thickness on the photostimulable phosphor (PSP) plate system; (c): 2 mm thickness on CMOS; (d): 2 mm thickness on PSP. Vertical black lines indicate that the mean values have no statistically significant differences from each other when analyzed using a Tukey test (*p>0.05.)

significantly higher radiopacity values than the PSP system, independent of the material thickness (p=0.036).

DISCUSSION

The radiopacity of a restorative material is a valuable diagnostic tool for evaluating the quality and long-term success of restorations. The radiographic diagnosis of recurrent caries, inadequate proximal contours, and marginal adaptation can be accurately interpreted because of the proper contrast between the enamel/dentin and the restorative material. Marginal defects and secondary caries are usually positioned on the gingival third of Class II restorations.[17] The first increment of the restorative material must be adequately radiopaque to be able to clearly evaluate the tooth-restoration interface.[4] It is desirable for resin composites to have a radiopacity equal to or greater than that of the enamel.[17] Materials with a radiopacity that is less than that of the enamel are not recommended for clinical usage in areas that are prone to secondary caries, especially as an initial increment material in cavities.[4]

Based on our results, the null hypothesis must be rejected because the radiopacities of the bulk-fill restoratives were significantly different. All of the composite-based bulk-fill restoratives showed higher radiopacity values than the enamel and dentin. Only Equia Fil, a glass ionomer–based material, showed similar radiopacity values to enamel; the radiopacity values of Equia Fil were higher than those of dentin. There are no previous studies in the dental literature that compared bulk-fill restorative radiopacities, although two of the bulk-fill restoratives in our study, Quixfil and SDR Bulk Fill, were compared with conventional composites in studies by Dukic and others[18] and Lachowski and others.[3] They reported radiopacities of 4.26 and 3.11 mm Al at 1-mm thickness on CCD for Quixfil and SDR Bulk Fill, respectively, whereas the present study showed 3.98 mm and 2.92 mm Al, respectively using CMOS and 3.55 mm and 2.34 mm Al, respectively, using PSP. The purity of the aluminum, methods used for evaluation, and thicknesses of the specimens are among the important factors causing variability in radiopacity.[19]

The composition of the material seems to be the most important factor that influences radiopacity.[20] The radiopacity of a material increases with a higher percentage of filler and larger amounts of elements with high atomic numbers in the filler particles.[21,22] Therefore, the manufacturers include chemical elements, such as barium, zinc, aluminum, strontium, silicon, yttrium, ytterbium, and lanthanum, in their products to increase radiopacity.[8] The higher the atomic number of the element added to the radiopaque filler, the higher the radiopacity of the material, because the absorption capacity of x-rays is increased.[3] The radiopacity of a dental composite material will exceed that of human enamel[22] if the filler volume is increased to 70% or beyond, and the amount of radiopaque oxide in filler particles is >20%.[21]

According to our results, Filtek Bulk Fill and SDR Bulk Fill, which have lower weight and volume percentages, showed lower radiopacity values compared with other materials. Tetric N-Ceram Bulk

Fill, Tetric EvoCeram Bulk Fill, Quixfil, and X-tra fill composite materials have high filler percentages; they also include radiopaque fillers that are composed of elements with higher atomic numbers in the filler composition, which leads to significantly higher radiopacity values compared with the other bulk-fill restoratives and enamel. The compound fluoro alumino silicate glass, which is a radiopaque filler used in the glass ionomer–based Equia Fil, did not provide an adequate amount of radiopacity, although the other fillers did. The ISO has stated that a resinous dental material should be at least as radiopaque as the same thicknesses of pure aluminum,[6] and the ADA recommends that these materials have a radiopacity equivalent to 1 mm Al, which is approximately equal to that of natural tooth dentin.[23] Some authors have suggested that a radiopacity equal to or slightly greater than enamel is more appropriate to detect secondary caries in posterior teeth.[24] It has been reported that highly radiopaque materials may mask caries because of superimposition. Moreover, a high radiopacity near a less radiopaque area can cause the Mach band effect, which produces a visual illusion that enhances the contrast between a lighter and a darker area, making the dark borderline area appear darker. This effect might be misinterpreted as caries.[25] In the current study, the radiopacities of Quixfil, X-tra fill, Tetric N-Ceram Bulk Fill, Tetric Evoceram Bulk Fill, and X-tra Base far exceeded the radiopacity of enamel, making them less suitable because excessive radiopacity may obscure the presence of a caries lesion.

According to recent literature, the radiopacities of resin composites, regardless of viscosity, exhibit huge variations. Independent of the radiographic system and evaluation method, radiopacity values of resin composites for 1-mm thickness are in the range of 1.7-3.5 mm Al,[3] 0.74-4.73 mm Al,[18] 1.29-4.63 mm Al,[25] and 1.50-3.88 mm Al,[25] similar to the values obtained in our study. Considering the results of previous reports and those of the current study, it may seem that the radiopacities of bulk-fill restoratives do not differ from those of incrementally filled conventional resin composites. Additionally, the present study showed that the increased thicknesses of bulk-fill restoratives improved their radiopacity values; this was also found by Lachowski and others[3] and by Pires de Souza and others.[26]

Direct systems have been found to be superior to PSP systems and a conventional system according to study by Wicht and others.[11] Comparison of direct digital sensors considering their advantages and disadvantages is controversial because CMOS receptors have only recently become available for x-ray use.[27] CCDs for x-ray imaging are a very stable and mature industry. They are popular because of their large detector format, high spatial resolution, good quantum efficiency, and nearly Fano-limited energy resolution. However, CCDs have pile-up limitations, problems associated with radiation damage, and high power requirements that become especially serious for long-lived, high-throughput x-ray missions. On the other hand, CMOS receptors are less sensitive to radiation damage, reduce power consumption, offer low costs, and produce a nondestructive and simple readout.[28] Theoretically, CMOS sensors are less efficient at gathering light and x-rays and, thus, have a lower quantum efficiency than CCDs. This means they gather less x-ray or light photon information and thus may not have as much diagnostic information to display. To compensate for reduced x-ray gathering, the CMOS sensors have microlenses and scintillators bonded to them to gather more light.[27]

The results of our study show that the radiopacity values of bulk-fill restoratives were significantly affected by different digital radiography methods, which agrees with the findings of Wicht and others.[11] who demonstrated that a CMOS sensor was the only radiographic system to show the space between the post and the dentin. This result might be an effect of the digital system automatic image processing, which could not be switched off, and of the high spatial resolution of the CMOS sensor. These findings are supported by Shi and others[29] who reported that CMOS sensors had increased perception for low-contrast details and were more sensitive than CCD.

Varying the radiographic exposure time and target distance are factors that affect the radiopacity of restorative materials.[7,30] Nevertheless, Gu and others,[5] using a digital x-ray system, reported that varying exposure times did not significantly affect the radiopacities of three typical dental products measured at a target distance of 30 cm, and varying target distance did not significantly affect the radiopacity as long as the samples were properly exposed. However, different radiography systems may require different exposure times and target distances.[13] From a theoretical standpoint, although an underexposed image has a background fog, overexposed images black out objects of low radiopacity.[5] In the present study, the exposure time was long enough so that not much background fog was produced and 1 mm of the aluminum step wedge was

visualized. Further studies on the radiopacities of bulk-fill restoratives are necessary to evaluate the effect of different exposure time and target distance combinations with different x-ray systems.

CONCLUSIONS

All of the bulk-fill restoratives that were tested passed the ISO and ANSI/ADA requirements for radiopacity. There were no differences in radiopacity between conventional composites and the bulk-fill materials. Varying thicknesses of bulk-fill restoratives affected their radiopacities. The radiopacity values of the CMOS system were found to be higher than those of the PSP system.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Izmir Katip Celebi Non-Interventional Clinical Studies Institutional Review Board. The approval code for this study is 201404750. This study was conducted by Sifa University, Department of Restorative Dentistry.

Conflict of Interest

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

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Effect of Photoactivation Timing on the Mechanical Properties of Resin Cements and Bond Strength of Fiberglass Post to Root Dentin

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Clinical Relevance

Delayed photoactivation may be beneficial for the clinical behavior of resin cements used to lute fiber posts. The root canal region is still a critical factor for the mechanical behavior of dual-cure resin cements.

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SUMMARY

Objectives: This study tested the hypothesis that photoactivation timing and resin cement affect mechanical properties and bond strength of fiberglass posts to root dentin at different depths.

Methods: Fiberglass posts (Exacto, Angelus) were luted with RelyX Unicem (3M ESPE), Panavia F 2.0 (Kuraray), or RelyX ARC (3M ESPE) using three photoactivation timings: light curing immediately, after three minutes, or after five minutes. Push-out bonding strength, PBS (n=10) was measured on each root region (coronal, middle, apical). The elastic modulus (*E*) and Vickers hardness (VHN) of the cement layer along the root canal were determined using dynamic indentation (n=5). A strain-gauge test was used to measure post-gel shrinkage of each cement (n=10). Residual shrinkage stress was assessed with finite element analysis. Data were analyzed with two-way analysis of variance in a split-plot arrangement and a Tukey test (α=0.05). Multiple linear regression analysis was used to determine the influence of study factors.

Results: The five-minute delay photoactivation timing significantly increased the PBS for all resin cements evaluated. The PBS decreased significantly from coronal to apical root canal regions. The mean values for *E* and VHN increased significantly with the delayed photoactivation for RelyX Unicem and decreased from coronal to apical root regions for all resin cements with the immediate-curing timing.

Conclusions: The PBS of fiber posts to root dentin, *E*, and VHN values were affected by the root canal region, photoactivation timing, and resin cement type. Shrinkage stress values decreased gradually with delayed photoactivation for all the cements.

INTRODUCTION

The esthetic and functional rehabilitation of endodontically treated teeth with less than two residual coronal walls usually requires the use of intraradicular posts.[1] Laboratory and clinical evaluations support the use of fiberglass posts for the retention of direct and indirect restorations.[2-6] Fiber posts have many advantages compared with metallic posts, especially the possibility of bonding to resin cements,[7] and have an elastic modulus (*E*) similar to dentin, which results in a lower stress concentration at the root, reducing the risk of root fractures.[4,6-8]

Several longitudinal assessments of fiber posts have shown a high success rate.[2,5,9-11] Most clinical failures were found to be related to debonding of the posts.[2,3,5,9-11] The fracture of the fiber post is also a commonly observed failure, mostly when used for restoring teeth without a ferrule.[10] The debonding occurred at the resin cement-dentin and/or resin cement-post interfaces.[12] Improving the adhesion at these interfaces will enhance the clinical performance of endodontically treated teeth restored with fiber posts.[13-16] The interface between resin cement and dentin is crucial for the retention of fiber posts.[15,17] The bonding at this interface can be influenced by several factors such as the root canal region, use of activators for conventional resin cements,[18] and properties and degree of conversion of the resin cements.[12,19-23]

Dual-cure resin cements have been widely used and were investigated in a number of clinical studies.[2,3,5] A chemical-cure is expected to adequately polymerize the cement in areas that cannot be entirely reached by a curing light, as well as in completely obscured regions. Despite the presence of some light, it may still not be enough for some dual-cure resin cements to reach an adequate degree of conversion.[12,20,21,23] Photoactivation has been recommended by manufacturers for different times after manipulation and insertion of the resin cement into the root canal.[22] Conventional dual-cure resin cements showed an increased degree of conversion after light curing, but the time elapsed from cement manipulation to light exposure had no influence on degree of conversion of the resin cement.[21,24] Self-adhesive resin cements were introduced in 2001 to simplify luting procedures and have been a good option to fix fiberglass posts due to their bonding performance and low viscosity.[25-28] No studies have assessed the influence of the time elapsed between the manipulation of resin cements and the light curing on the mechanical properties and bond strength of these cements for luting fiberglass posts.

Based on the bonding mechanism and the importance of pH-buffering for polymerization, a time delay between the cement mixing and photoactivation steps may favor the ability of dual-curing resin cement to bond to dentin.[29,30] A rapid increase of cement viscosity by light irradiation[31] may hinder the reaction of the acidic monomers with the dental tissues, which may affect the bonding mechanism.[30] Additionally, the delay of dual-cured resin cement photoactivation can reduce polymerization stress.[30] Another aspect that can affect the mechanical properties of resin cements and the bond strength of a post is the polymerization shrinkage of resin cements. Shrinkage of the cement can lead to considerable residual shrinkage stresses in the cement at root canal walls and at the interfaces. The composition of resin cements and different light curing may affect shrinkage stress generated in the root canal. [30]

The aim of this study was to test the influence of the photoactivation timing and the resin cement on the mechanical properties and bond strength of fiber posts to root dentin at different depths. The null hypothesis was that the mechanical properties and bond strength of the resin cements are not influenced by photoactivation timing, resin cement, or their location in the root canal.

METHODS AND MATERIALS

Specimen Preparation

A total of 135 freshly extracted bovine incisors with straight roots and canals were selected for this study and used within one month after extraction. The crowns were removed to obtain a root length of 15 mm. A working length of 14 mm (ie, 1 mm above the

apex) was established. The root canals were instrumented by using No. 70 stainless steel K-files in apical thirds (Dentsply Maillefer, Ballaigues, Switzerland) and Gates-Glidden (No. 4 and No. 5; Dentsply Maillefer) in middle and coronal thirds. All instrumentation procedures were followed by 1.0% sodium hypochlorite (NaOCl) irrigation.

Post space preparations were performed in the root canals at a 10-mm depth using conical burs included in the fiberglass post kit (Exacto No. 3; Angelus, Londrina, Brazil). The burs were replaced after every five preparations. The prepared root canals were cleaned thoroughly with tap water and gently dried with absorbent paper points. The fiber posts were luted using one of three dual-cure resin cements: a self-adhesive resin cement, RelyX Unicem (3M ESPE, St Paul, MN, USA) or one of two conventional resin cements, Panavia F 2.0 (Kuraray, Okayama, Japan) with a specific adhesive system for Panavia F, and RelyX ARC (3M ESPE) with the Adper Scotchbond Multi-Purpose adhesive system (3M ESPE) used with the dual-cure mode. The adhesive systems were manipulated and cured following the manufacturers' instructions (Table 1). The posts were etched by immersion in 24% hydrogen peroxide for one minute,[15] rinsed with water for one minute, and dried with an air stream followed by the application of a silane coupling agent for one minute (Silano, Angelus). Resin cements were handled according to the manufacturers' instructions and inserted manually into the root canal with No. 40 stainless steel K-files (Dentsply Maillefer). The fiberglass posts were placed into the root canals with light digital pressure, and the resin cements were photoactivated using one of three different timings (n=15): Immediate (photoactivation immediately after resin cement manipulation, insertion, and excess removal); three minutes (photoactivation delayed for three minutes after the steps described previously); and five minutes (photoactivation delayed for five minutes after the steps described previously). For the three- and five-minute groups the specimens were stored in a dark box. All roots were covered externally with wax to avoid lateral polymerization. Photoactivation was performed through the coronal portion of the root at the buccal, lingual, and coronal faces for 40 seconds, for a total of 120 seconds of light exposure. The photoactivation procedures were performed using a quartz-tungsten-halogen light-curing unit with a 600 mW/cm^2 output (Optilux 501, Demetron Kerr, Orange, CA, USA). The output was periodically measured during the study. After post cementation, the specimens were stored in distilled water at 37°C for 24 hours in a dark plastic container.

Push-out Test

From the 15 specimens prepared for each group, 10 root specimens were randomly selected for "thin-slice" push-out bond strength tests. The roots were sectioned with a precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) into six 1.0-mm-thick slabs to obtain two slices each from the coronal, middle, and apical thirds. For the push-out test, the load was applied using a cylindrical tip attached to a mechanical testing machine (DL 2000, EMIC, São José dos Pinhais, Brazil). The diameters of the testing tips (1.5, 1.2, and 0.9 mm) and bases (2.5, 2.2, and 2.0 mm) were selected according to the origin of each slice to accommodate the conical design of the posts and ensure shear stresses along the bonded interface.[32] The load was applied in the apical-coronal direction of the specimens at a crosshead speed of 0.5 mm/min until failure. The bond strength (in MPa) of the post to the root segment was calculated by dividing the load at failure (in N) by the interfacial surface area A of the post section (in mm^2). The lateral surface area of the conical section was calculated using the following formula:

$$A = 2\pi[(R + r)/2]h$$

where $\pi = 3.14$, R is the coronal post radius, r is the apical post radius, and h is the root-slice thickness. The measurements of the root-slice thickness were made using a digital caliper with 0.01-mm accuracy (Mitutoyo, Tokyo, Japan). The radii of the post sections were obtained by capturing an image through a stereomicroscope (Mitutoyo) with a digital camera (Moticam 2300, Motic, Richmond, BC, Canada) and measured with image analysis software (Motic Image Plus 2.0, Motic). The specimens were examined under the stereomicroscope to determine whether the failure mode was adhesive between dentin and post, cohesive in the cement, or mixed.

Vickers Hardness and *E* Determination

Five root specimens from each group were used for determining the *E* and Vickers hardness (VHN) of the cement layer at various levels of the root. The root was sectioned longitudinally into two halves using a precision saw (Isomet 1000, Buehler). One half of each root was randomly selected and embedded in polyester resin (Instrumental Instrumentos de Medição Ltda, São Paulo, Brazil). The surfaces were finished using silicon-carbide papers

Table 1: *Main Composition of the Resin Cements Used in This Study*

Material	Manufacture	Batch Number	Instructions for Use	Composition
RelyX Unicem	3M ESPE, St Paul, USA	421172	1) Dispense cement onto a mixing pad and mix for 20 s; 2) Apply cement in and around canal using endodontic file.	Base: glass powder, methacrylated phosphoric acid esters, TEGDMA, silane-treated silica, sodium persulfate; Catalyst: glass powder, substituted dimethacrylate, silane-treated silica, sodium p-toluenesulfonate, calcium hydroxide
Panavia F 2.0	Kuraray, Okayama, Japan	51213	1) Dispense and mix immediately one drop each of ED PRIMER II; 2) Apply the ED PRIMER into the root canal with a disposable brush tip; 3) After 30 seconds, remove the excess with a vacuum aspirator; 4) Mix sufficient Paste A and Paste B on the paper pad for 20 s; 5) Insert Panavia F manually into the root canal with an endodontic file.	Paste catalyst: Bis-GMA; TEGDMA; glass filler Paste A: silanated silica filler; silanated colloidal silica; MDP; hydrophilic aliphatic D; hydrophobic aliphatic D; dl-camphorquinone; catalysts; initiators Paste B: silanated Ba glass; sodium fluoride; hydrophilic aromatic D; hydrophobic aliphatic D; catalysts; accelerators; pigments (filler content $\cong$ 76%)
RelyX ARC	3M ESPE, St Paul, USA	N140749	1) Acid etch with 35% phosphoric for 15 s; 2) Rinse with water for 15 s and air dry for 2 s; 3) Remove excess moisture with a paper point; 4) Apply activator of the adhesive system in canal and remove excess with air drying (5 s); 5) Apply primer of the adhesive system in canal and remove excess by air drying (5 s); 6) Apply catalyst of the adhesive system in canal; 7) Dispense cement onto a mixing pad and mix for 10 s; 8) Apply cement in and around canal using endodontic file.	Bis-GMA, TEGDMA, pigments, amine, benzoyl peroxide and zirconia silica (filler content $\cong$ 67.5%, size $<$ 1.5 um).
Exacto Fiber Post	Angelus, Londrina; Brazil	8214	1) Etch the posts by immersion in 24% H_2O_2 for 1 min; 2) Rinse with water for 1 min; 3) Dry with an air stream; 4) Apply of a silane-coupling agent for 1 min.	Opaque fiberglass post, fiberglass (87%), epoxy resin (13%).

Abbreviations: Ba, barium; Bis-GMA, bisphenol A glycidyl methacrylate; H_2O_2, hydrogen peroxide; MDP, 10-methacryloxydecyl dihydrogen phosphate; TEGDMA, triethylene glycol dimethacrylate.

(600-, 800-, 1200-, and 2000-grit; Norton, Campinas, Brazil) for one minute each and polished with metallographic diamond pastes (6, 3, 1, and 0.25 μm; Arotec, São Paulo, Brazil) for one minute each. The specimens were cleaned using an ultrasound bath with distilled water for 10 minutes after use of each diamond paste. The *E* and VHN of the resin cement layer were assessed with a microhardness indenter (CSM Micro-Hardness Tester, CSM Instruments, Peseux, Switzerland), 24 hours after luting of the fiber posts. The indentations were made at 1.0-mm intervals, starting at 0.5 mm from the coronal surface (coronal third) and ending at 8.5 mm (apical third). The testing procedure was carried out under load control. The load was increased at a constant rate from 0 to 500 mN in 20 seconds (1.5 N/min). The maximum force of 500 mN was applied for five seconds, then the force was gradually decreased to 0

Table 2: *Results for Bond Strength in MPa (n=10)*[a]

Resin Cement	Timing of Light Activation		
	Immediate	3 Min	5 Min
RelyX Unicem	7.6 (3.4) Ac	13.6 (3.9) Ab	18.8 (6.6) Aa
Panavia	6.1 (1.9) Ab	9.5 (3.5) Ba	11.0 (4.0) Ba
RelyX ARC	5.5 (2.5) Ac	9.3 (3.4) Bb	12.2 (4.1) Ba

[a] *Different letters (lowercase for moment of light activation [columns], uppercase for cement [rows]) indicate statistical difference (p<0.05).*

mN in 20 seconds.[12] The load and the penetration depth of the indenter were continuously measured during the load-unload cycle.

Universal hardness is defined as the test force divided by the apparent area of the indentation at maximal force. From a multiplicity of measurements stored in the manufacturer-supplied database, a conversion factor between universal hardness and VHN was calculated to determine the VHN. The indentation modulus was determined from the tangent of the indentation-depth curve at maximal force. The calculated indentation modulus is comparable with the E of the material.[12]

Post-gel Shrinkage Measurements

Resin cement post-gel linear shrinkage was determined[33,34] using the strain gauge method (n=10). The resin cements (RelyX Unicem, Panavia F 2.0, and RelyX ARC) were manipulated and placed on top of a biaxial strain gauge (CEA-06-032WT-120, Measurements Group, Raleigh, NC, USA) that measured strains during polymerization in two perpendicular directions. The resin cement was polymerized using a quartz-tungsten-halogen unit (Optilux 501, Demetron Kerr) with the light tip placed 1 mm from the surface of the cement. The radiant exposure was set at 24 J/cm^2 (600 mW/cm^2 × 40 seconds). A strain conditioner (ADS0500IP, Lynx Tecnologia Eletrônica, São Paulo, Brazil) converted electrical resistance changes in the strain gauge to voltage changes through a quarter-bridge circuit with an internal reference resistance. Post-gel strains resulting from polymerization shrinkage were monitored for 10 minutes, starting from the beginning of photoactivation. The two perpendicular strain recordings were averaged because the material properties can be assumed to be homogeneous and isotropic on a macro scale. The post-gel shrinkage value at 10 minutes was used in the finite element analysis (FEA).[34] The mean shrinkage strain, which is the linear shrinkage, was reported as percentage volumetric shrinkage by multiplying three times the linear shrinkage by 100%.

Residual Stress Calculation—FEA

To evaluate corresponding residual shrinkage stresses in the root canal, a finite element simulation was carried out using an axisymmetric root restoration model (Figure 1). The geometric model was based on a digitized bucco-palatal cross-section of a maxillary central incisor with cemented fiber post. Coordinates were obtained using ImageJ software (National Institutes of Health, Bethesda, MD, USA). A simplified boundary condition was assumed at the cutting plane of the root (zero-displacement in horizontal and vertical directions). In all models, the root canal was considered as being not root filled (ie, no gutta-percha and sealer) and the posts were considered to be perfectly bonded. The E of dentin[35] was 18.6 GPa and the Poisson ratio was 0.31; the E of the periodontal ligament[36] was 1.18 MPa and the Poisson ratio, 0.45; the E of the bone[35] was 1.37 GPa and the Poisson ratio, 0.30; and the E of the post[37] was 9.5 GPa and the Poisson ratio, 0.34. The E values of the three resin cements using the three photoactivation timings at nine depths were obtained experimentally with the indentation test, as previously described. The Poisson ratio was chosen to be the same[38] for all resin cements at 0.30.

Table 3: *Results of Bond Strength (in MPa) for Each Resin Cement (n=10)*

Root Third	RelyX Unicem				Panavia		
	Immediate	3 Min	5 Min	Pooled Average	Immediate	3 Min	5 Min
Coronal	9.7 (3.8)	16.5 (4.3)	23.6 (6.6)	16.6 (7.6) A[a]	7.8 (1.8) Ab	12.0 (3.1) Aa	14.6 (2.3) Aa
Middle	7.3 (2.7)	13.5 (2.3)	19.3 (5.6)	13.4 (6.1) B	6.0 (1.4) Bb	10.1 (2.9) Ba	11.3 (3.1) Ba
Apical	6.0 (2.7)	10.7 (2.5)	13.5 (3.1)	10.1 (4.1) C	4.6 (1.0) Cb	6.4 (2.0) Cab	7.3 (2.2) Ca
Pooled average	7.6 (3.4) c	13.6 (3.9) b	18.8 (6.6) a		The interaction between factors was significant ($p<0.001$).		

[a] *Different letters (lowercase for activation mode [columns], uppercase for root third [rows]) indicate statistical differences (p<0.05). In the absence of significant interaction, the comparison is performed for pooled averages.*

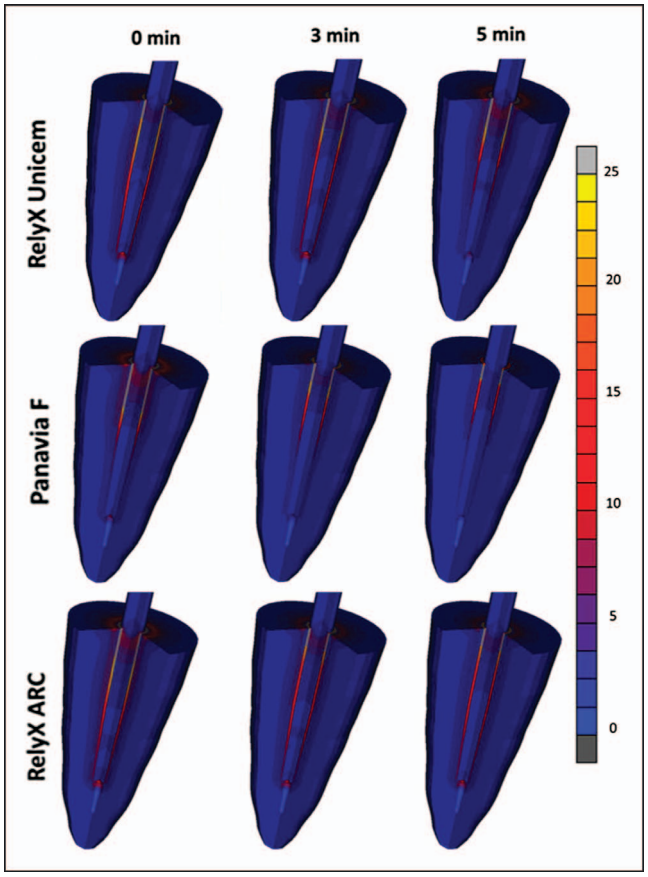

Figure 1. *Residual shrinkage stress distribution (modified von Mises equivalent stress, MPa) for the three resin cements and three photoactivation timings calculated by the numerical polymerization model.*

The FEA was performed using MSC.Mentat (preprocessor and postprocessor) and MSC.Marc (solver) software (MSC Software Corporation, Santa Ana, CA, USA). Nine FEA models were generated with three resin cements (RelyX Unicem [3M ESPE], Panavia F 2.0 [Kuraray], and RelyX ARC [3M ESPE]) and the three photoactivation timings (light curing immediately, three minutes, and five minutes). Polymerization shrinkage was simulated by thermal analogy. Temperature was reduced by 1°C, and the linear shrinkage value (post-gel shrinkage) was entered as the coefficient of thermal expansion. The linear shrinkage values for the coronal, middle, and apical portions of the resin cement were determined by the correlation with the E at each depth.

Modified von Mises equivalent stress, which accounts for the difference in compressive and tensile strengths of the dentin and resin cements, was used to express the residual shrinkage stress conditions in the resin cement nodes at the post and dentin interfaces. Because the finite element program averaged stresses across interfacial nodes, the cement was isolated from the post and the dentin before the nodal values were recorded. Furthermore, stress values were obtained at nodes of the resin cement that corresponded to the nine depths where the E and post-gel shrinkage values were obtained in the experimental test.

Statistical Analysis

Given that push-out bond strength, E, and VHN data were normally distributed (Shapiro-Wilk, $p>0.05$), equality of variance values (Levene test, $p>0.05$) were verified for the push-out bond strength, E, VHN, and post-gel shrinkage before parametric statistical tests were performed. Two-way analysis of variance (ANOVA) in a split-plot arrangement and Tukey test ($\alpha=0.05$) were used to compare push-out bond strength, E, and VHN with the plot represented by the photoactivation timing, resin cement, and their interaction and the subplot represented by the root regions. The post-gel shrinkage values were statistically analyzed by two-way ANOVA followed by Tukey honestly significant difference post-hoc tests ($p=0.05$). The data of failure mode were subjected to the χ^2 test ($p=0.05$). A multiple linear regression analysis was used to determine the influence of the photoactivation timing and root canal third on the bond strength

Table 3: *Results of Bond Strength (in MPa) for Each Resin Cement (n=10) (ext.)*

Root Third	RelyX ARC			
	Immediate	3 Min	5 Min	Pooled Average
Coronal	7.2 (2.6)	12.0 (3.4)	14.8 (3.4)	11.3 (4.4) A
Middle	5.3 (2.2)	9.1 (2.5)	12.5 (3.9)	8.9 (4.1) B
Apical	4.0 (1.6)	6.8 (2.5)	9.2 (3.3)	6.7 (3.2) C
Pooled average	5.5 (2.5) c	9.3 (3.4) b	12.2 (4.1) a	

Table 4: *Percentage of Failure Mode Distribution (%)*

Photoactivation Timing	Resin Cement	Root Region								
		Coronal			Middle			Apical		
		A	M	C	A	M	C	A	M	C
Immediate	RelyX Unicem	60	40	0	65	30	5	75	15	10
	Panavia F	80	20	0	80	15	5	90	0	10
	RelyX ARC	75	25	0	80	20	0	95	0	5
3-min delay	RelyX Unicem	55	45	0	60	40	0	75	15	0
	Panavia F	70	30	0	75	25	0	90	0	5
	RelyX ARC	75	25	0	70	30	0	95	5	0
5-min delay	RelyX Unicem	50	50	0	50	50	0	75	15	10
	Panavia F	70	30	0	75	25	0	90	0	10
	RelyX ARC	60	40	0	75	25	0	95	0	5

Abbreviations: A, adhesive failure between dentin and post; C, cohesive failure in cement; and M, mixed failure.

and also to determine the influence of the photoactivation timing and root canal depth (0.5 to 8.5 in 1.0 mm intervals) in *E* and VHN data (SAS 9.1 software package, SAS, Cary, NC, USA). The level of significance was set at $\alpha=0.05$.

RESULTS

Push-out Bond Strength

Mean push-out bond strengths are shown in Table 2 and Table 3. A two-factor ANOVA showed that mean push-out strengths were significantly affected by the factors resin cement ($p<0.001$), photoactivation timing ($p<0.001$), and root region ($p<0.001$). There were significant interactions between the factors resin cement and root region ($p<0.001$) and between the factors photoactivation timing and root region ($p=0.002$); however, the interaction between resin cement and photoactivation timing was not statistically significant ($p=0.303$), nor was the interaction among the photoactivation timing, resin cement, and root region ($p=0.700$).

For the immediate photoactivation timing, all resin cements resulted in statistically similar mean push-out bond strengths, irrespective of the root region ($p=0.651$). For the three- and five-minute photoactivation timings, RelyX Unicem showed significantly higher mean push-out bond strengths than other resin cements, irrespective of the root region ($p<0.001$). Comparing the mean push-out bond strengths within each resin cement, RelyX Unicem had significantly higher mean push-out bond strengths for the three- and five-minute photoactivation timings than the immediate curing ($p<0.001$). RelyX ARC and Panavia F cements had significantly higher mean push-out bond strengths for the five-minute timing than for the immediate curing ($p<0.001$). The three- and five-minute photoactivation timings of the Panavia F resin cement had statistically similar mean push-out bond strengths ($p=0.303$).

The multiple linear regression test demonstrated that mean push-out bond strength was significantly associated with the photoactivation timing ($p<0.001$) and root canal depth ($p<0.001$) for all resin cements (Figure 2).

The distribution of failure pattern for each experimental group is shown in Table 4. For all resin cements and photoactivation timings, the adhesive failures were significantly more prevalent at the apical root region and the mixed failures were more prevalent at the coronal root region.

Hardness and *E* Measurements

Hardness results are shown in Table 5 and Table 6. VHN was significantly affected by the resin cement type ($p<0.001$), photoactivation timing ($p<0.001$), and root regions ($p<0.001$). There were significant interactions between resin cement and root region ($p<0.001$), between photoactivation timing and resin cement type ($p=0.002$), and among resin cement type, photoactivation timing, and root region ($p=0.014$). However, the interaction between photo-

Table 5: *Mean (SD) of Vickers Hardness in N/mm²*

Resin Cement	Timing of Light Activation		
	Immediate	3 Min	5 Min
RelyX Unicem	40.2 (7.9) ABb[a]	49.2 (2.7) Aa	60.1 (2.8) Aa
Panavia	36.3 (0.8) BCa	36.8 (1.3) Ba	39.1 (2.6) Ba
RelyX ARC	46.1 (1.5) Aa	50.4 (1.3) Aa	51.1 (0.7) Aa

[a] *Different letters (lowercase for moment of light activation [columns], uppercase for cement [rows]) indicate statistical difference (p<0.05).*

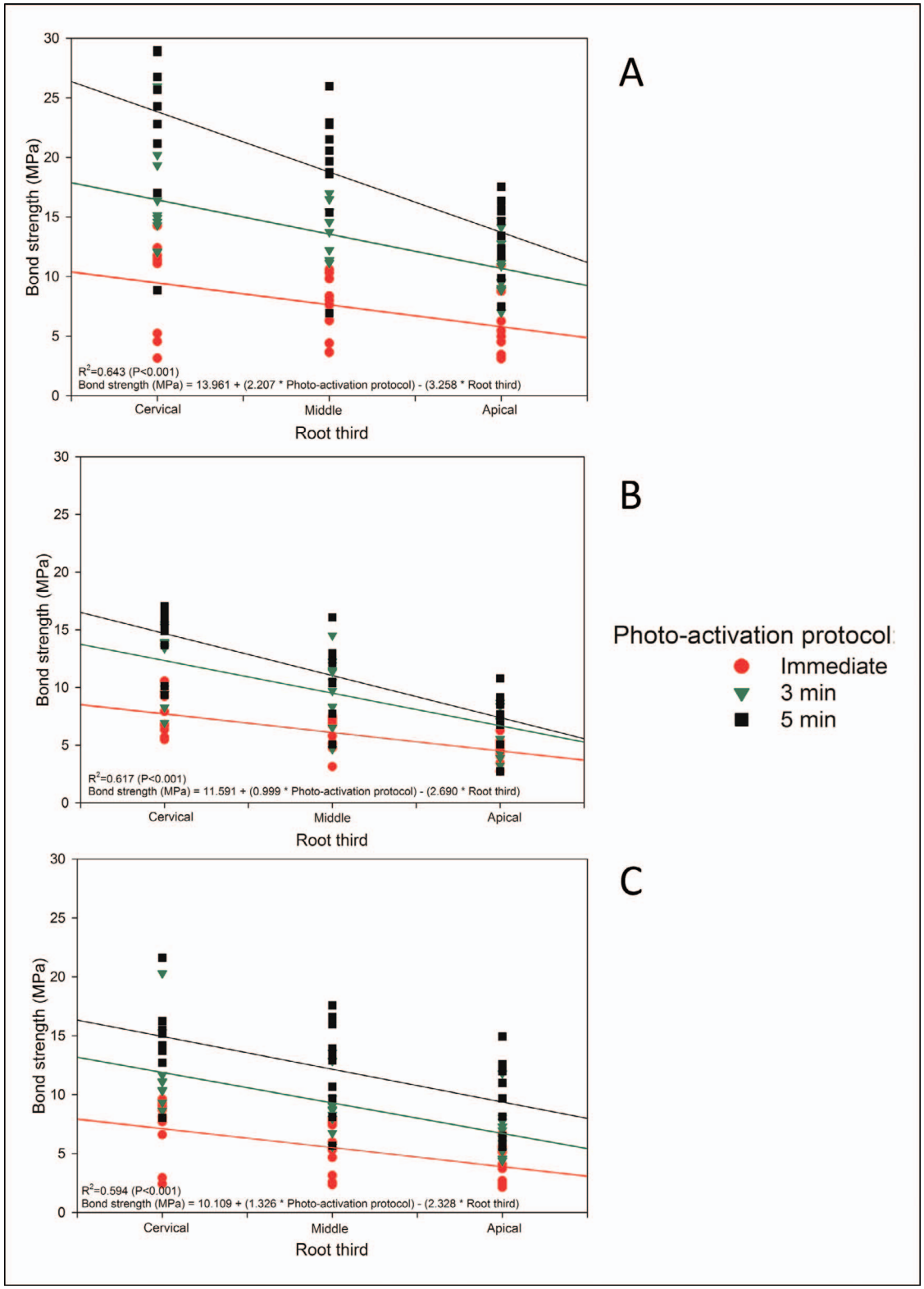

Figure 2. *The relationship between bond strength in root canal and photoactivation timing and root regions. (A): RelyX Unicem, (B): Panavia F, and (C): RelyX ARC.*

Table 6: *Mean (SD) of Vickers Hardness in N/mm^2 for Each Resin Cement (n=5)*

Root Third	RelyX Unicem				Panavia			
	Immediate	3 Min	5 Min	Pooled Average	Immediate	3 Min	5 Min	Pooled Average
Coronal	58.8 (11.2)	66.0 (4.5)	75.5 (4.8)	66.8 (9.7) A[a]	54.8 (3.6)	58.9 (1.3)	64.4 (3.2)	59.4 (5.5) A
Middle	36.0 (6.1)	46.8 (1.9)	62.8 (5.7)	48.5 (12.4) B	35.6 (4.4)	34.8 (2.3)	34.8 (3.3)	35.1 (3.0) B
Apical	25.7 (7.8)	34.6 (2.5)	42.1 (8.9)	34.2 (9.3) C	18.6 (3.3)	16.9 (0.9)	18.2 (1.8)	17.9 (2.1) C
Pooled average	40.2 (16.5) b	49.2 (14.0) b	60.1 (15.7) a		The mode of activation was not significant (p=0.531).			

[a] *Different letters (lowercase for activation mode [columns], uppercase for root third [rows]) indicate statistical differences (p<0.05). In the absence of significant interaction, the comparison is performed for pooled averages.*

activation timing and root region was not significant (p=0.738). In general, the VHN of Panavia F and RelyX ARC were not influenced by the photoactivation timing. RelyX Unicem showed significantly higher mean VHN for the five-minute photoactivation timing than the other two tested timings, irrespective of the root region. Mean VHN decreased gradually from the coronal third to the apical third, irrespective of resin cement.

E results are shown in Table 7 and Table 8. The mean *E* was significantly affected by the resin cement ($p<0.001$), photoactivation timing (p=0.007), and root region ($p<0.001$). There is significant interaction between resin cement and root region ($p<0.001$). However, the interactions between resin cement and photoactivation timing ($p<0.3225$), between photoactivation timing and root region ($p<0.700$), and among resin cement, photoactivation timing, and root region (p=0.310) were not significant. Mean *E* reduced gradually with root depth for all resin cements. In general, the mean *E* for Panavia F and RelyX ARC was not influenced by the photoactivation timing. However, the mean *E* of RelyX Unicem was significantly higher with the five-minute photoactivation timing.

The multiple linear regression test demonstrated that mean VHN and *E* were significantly associated with the photoactivation timing ($p<0.001$) and root canal depth ($p<0.001$) for all resin cements (Figures 3 and 4). The mean VHN was significantly associated with root canal depth for resin cements RelyX Unicem and Panavia F (β=−5.302 and −6.702, respectively). Photoactivation timing had significant correlation with VHN values for RelyX Unicem (β=3.913).

Mean *E* was significantly influenced by the root canal depth for the resin cement Panavia F (β=1.107), more than by the depths for RelyX Unicem and RelyX ARC (β=0.644 and 0.538, respectively). Photoactivation timing had significant influence on *E* for RelyX Unicem (β=0.519).

Table 7: *Mean (SD) of Elastic Modulus in Gigapascals (GPa)*

Resin Cement	Moment of Light Activation			Pooled Average
	Immediate	3 Min	5 Min	
RelyX Unicem	5.6 (0.4)	7.4 (0.6)	8.1 (1.1)	7.0 (1.5) A[a]
Panavia	6.5 (0.5)	7.4 (0.3)	7.7 (0.7)	7.2 (0.7) A
RelyX ARC	6.7 (1.2)	6.9 (0.5)	7.3 (0.6)	7.0 (0.8) A
Pooled average	5.8 (1.3) c	6.7 (1.0) b	7.6 (0.7) a	

[a] *For pooled averages, different letters (lowercase for moment of light activation [columns], uppercase for cement [rows]) indicate statistical difference (p<0.05).*

Post-gel Shrinkage

The mean values and standard deviations for the post-gel shrinkage of three resin cements and polymerization timings are presented in Table 6. Two-way ANOVA revealed that the volumetric shrinkage mean was significantly affected by the resin cement ($p<0.001$) and photoactivation timing ($p<0.001$). There was also a significant interaction between resin cement type and photoactivation timing (p=0.001). For immediate photoactivation, all resin cement showed statistically similar volumetric post-gel shrinkage. The post-gel shrinkage means decreased gradually with delayed photoactivation timing for RelyX and RelyX ARC, but more sharply with Panavia F. As such, the three- and five-minute photoactivation timings for RelyX Unicem and RelyX ARC had significantly higher volumetric post-gel shrinkage mean than those for Panavia F.

Residual Stress Calculation—FEA

Modified von Mises shrinkage stress distributions for all groups are shown in Figure 1. For all cements the immediate photoactivation timing generated higher shrinkage stress than the three- and five-minute timings. Panavia F had the lowest shrinkage stress at the five-minute photoactivation timing of the three resin cements. The delayed photoactivation timing lowered the overall stress levels in the root

Table 6: *Mean (SD) of Vickers Hardness in N/mm^2 for Each Resin Cement (n=5) (ext.)*

Root Third	RelyX ARC		
	Immediate	3 Min	5 Min
Coronal	61.0 (2.7) Aa	59.2 (2.8) Aa	58.5 (0.7) Aa
Middle	44.7 (4.4) Bb	50.1 (2.9) Bab	51.2 (2.5) Ba
Apical	32.6 (2.3) Cb	42.0 (2.1) Ca	43.5 (1.6) Ca
Pooled average	The interaction between factors was significant (p=0.013).		

canal, especially for RelyX Unicem with the five-minute delay.

DISCUSSION

The null hypothesis tested in this study was rejected. The results indicated that the retention of the fiber posts to the root canal dentin measured using the push-out strength was significantly influenced by the photoactivation timing, resin cement type, and root region.

Unlike clinical situations, the root canals in the present study were not filled with sealer and gutta-percha before post space preparation, aiming to eliminate a possible influence of contaminants.[16,39] Gutta-percha and sealers may affect the bond strength of self-adhesive[16] and conventional resin cements.[17,39] In such a case, the influence of the study factors tested would be masked by the presence of sealer and gutta-percha. The impact of the NaOCl and EDTA solutions on bond strength has been controversial in the literature. For conventional and self-adhesive resin cements, the use of NaOCl did not improve the bond strength[17]; however, the use of EDTA solutions can improve the bond strength of conventional resin cements,[17] but not self-adhesive cements.[29] Therefore, the influence of these factors was eliminated in the present study by rinsing the root canals with filtered water only after post preparation.[36,39]

In addition to the bond strength of fiber posts, we also measured the *E* and VHN of the cements within the root canals, which can provide valuable information indicative of clinical performance.[28] The hardness (VHN) of resin cements is related to degree of conversion when the same material is evaluated and is thus useful for comparing conversion of the resin cements submitted to different photoactivation timings.[40] The *E* is an important mechanical property that relates stress with functional deformation of materials. *E* is directly related to the VHN[12] and plays an important role in polymerization stress.[41] Therefore, *E* and VHN have a direct effect on the bond strength of resin cements used to lute fiber posts to the root canal dentin. *E*, VHN, and push-out bond strength values of all resin cements, irrespective of the photoactivation timing, decreased from coronal to apical region. The opaque fiber posts used in this study, as well as the translucent posts, do not transmit light along their full length.[12,42] This may have limited the polymerization of the resin cements in the middle and apical root thirds,[20,43,44] resulting in lower push-out bond strength,[7,12,] *E*, and VHN values in these regions,[12] mainly for Rely X ARC and Panavia F when compared with the self-adhesive resin cement RelyX Unicem.[44] Push-out bond strength and mean VHN measured in the coronal and middle thirds were higher than those measured in the apical third.[40] Thus, the luting of fiber posts with increased lengths should be treated with caution, mainly when the fiber post is cemented with RelyX ARC.[44] The limitation of polymerization in the deeper region of a root canal is reflected in a fracture resistance and structure deformation similar to that of root-treated teeth restored with shorter (5.0 or 7.5 mm) or longer (10.0 mm) posts.[4,46]

The delayed photoactivation timings increased VHN and *E* values for self-adhesive resin cement (RelyX Unicem) but not for conventional resin cements (Panavia F and RelyX ARC). However, the delayed photoactivation timings increased the push-out strength values for all resin cements evaluated (Figure 2) and reduced the post-gel shrinkage (Table 6). Taking into consideration that VHN and degree of conversion of resin cements are directly related,[40] it can be assumed that the RelyX ARC and Panavia F reached a similar degree of conversion using the immediate and delayed photoactivation timings. The improvement of mechanical properties for RelyX Unicem with delayed light curing may be explained by increased contact of the material with moisture of the root dentin during the curing process.[48] Water plays a critical role in the effectiveness of a self-adhesive resin cement's curing process and bonding characteristics.[48] Additionally, the longer duration

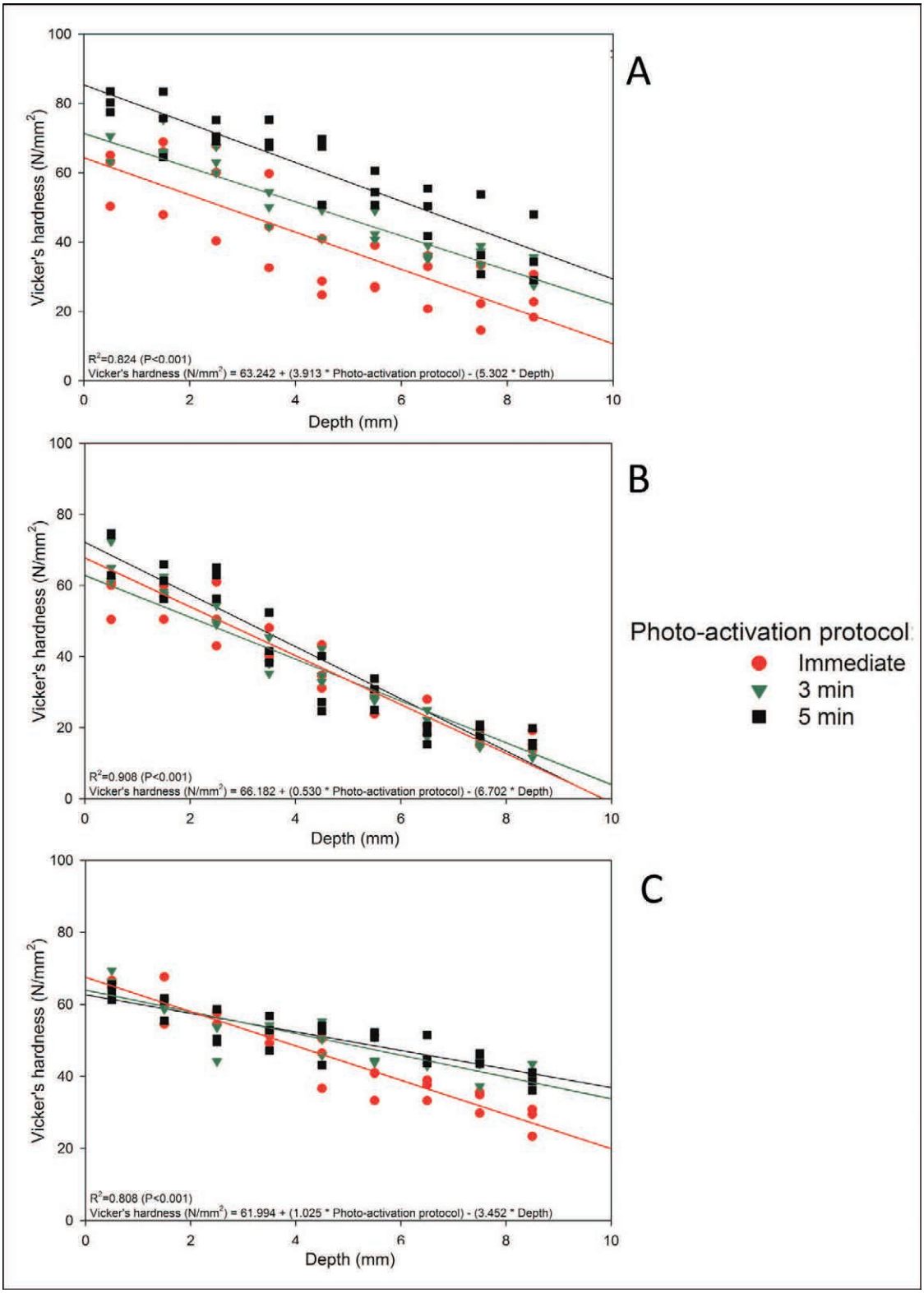

Figure 3. *The relationship between Vickers hardness in root canal and photoactivation timing and root canal depth. (A): RelyX Unicem, (B): Panavia F, and (C): RelyX ARC.*

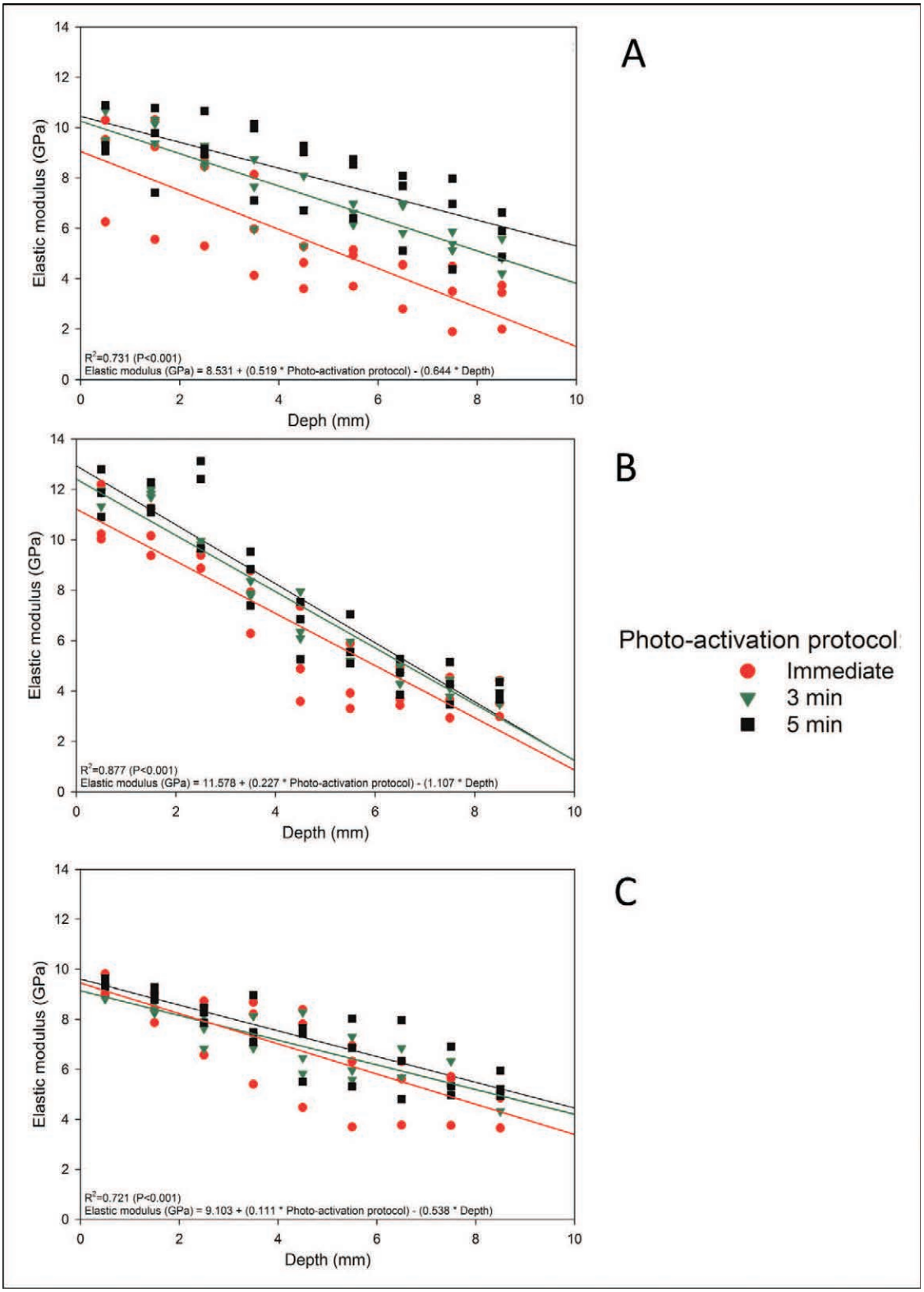

Figure 4. *The relationship between elastic modulus in root canal and photoactivation timing and root canal depth. (A): RelyX Unicem, (B): Panavia F, and (C): RelyX ARC.*

Table 8: *Mean (SD) of Elastic Modulus in Gigapascals (GPa) for Each Resin Cement (n=5)*

Root Third	RelyX Unicem				Panavia			
	Immediate	3 Min	5 Min	Pooled Average	Immediate	3 Min	5 Min	Pooled Average
Coronal	8.2 (2.2)	9.5 (0.5)	9.6 (1.2)	9.1 (1.4) A[a]	10.1 (0.6)	11.1 (0.2)	11.7 (1.1)	11.0 (0.9) A
Middle	5.1 (1.1)	6.9 (1.1)	8.4 (1.5)	7.4 (1.8) B	5.8 (1.3)	6.8 (0.5)	7.0 (0.7)	6.5 (1.0) B
Apical	3.4 (1.1)	5.6 (0.3)	6.4 (1.4)	8.1 (1.8) C	3.8 (0.8)	4.2 (0.2)	4.3 (0.6)	4.1 (0.6) C
Pooled average	The mode of activation was not significant (p=0.108).				The mode of activation was not significant (p=0.218).			

[a] *Different letters (lowercase for activation mode [columns], uppercase for root third [rows]) indicate statistical differences (p<0.05). In the absence of significant interaction, the comparison is performed for pooled averages.*

of chemical activation can be expected to provide a uniform polymerization at the bottom of deep areas where access for curing light is limited. Immediate light activation may limit the interactions of monomers, especially under constrained conditions found in a cavity.[50] Previous studies also showed that delayed photoactivation of RelyX ARC[21,46] and Panavia F[21] did not increase the degree of conversion. This performance can be explained by the presence a chemical activator in the adhesive systems that were used before these resin cements.[49,50] Although the degree of conversion was not affected by the curing timings, delaying photoactivation decreased post-gel shrinkage in this study and polymerization shrinkage stress in a previous study.[46] Lower polymerization shrinkage stress has been related to higher bond strength values.[38] Therefore, delayed photoactivation may not have increased the degree of conversion of the RelyX ARC and Panavia F resin cements, but it could have reduced their polymerization shrinkage stress and consequently increased their push-out strength. Given that most failures of fiber posts are related to debonding,[2,3,5] the higher bond strength found with the five-minute delayed timing is an important finding because it may reduce the incidence of fiber post debonding.

FEA can help to explain the performance of the resin cements cured with the three photoactivation timings because it allows us to study the impact of the resulting different material properties on the stress generation. Given that the FEA models all had the same post/root geometry, the same post/root properties, and the same cement bonding conditions, the stress in the root canal was determined by the combination of post-gel shrinkage and E of the resin cements. In this FEA, the E varied along the post according to location-dependent values that were measured experimentally; whereas, the post-gel shrinkage values for each time were applied along the entire post. The FEA shows that despite the similar or higher E values obtained with delayed photoactivation, residual shrinkage stress levels along the posts decreased. Although not modeled, post-gel shrinkage is likely to decrease the deeper the cement is located,[47] and thus farther down the root canal, the shrinkage stresses can be expected to reduce further. These FEA observations support the previous speculation that delayed photoactivation reduced residual shrinkage stresses and may improve fiber post retention in the coronal region.

The results of *in vitro* studies should be carefully interpreted before being extrapolated to a clinical context. However, the general response of the resin cements to delayed photoactivation timing should also apply under clinical conditions. Therefore, this study presents a potential strategy for clinicians to restore endodontically teeth with delayed photoactivation timing. The current analysis indicates that the best results were found when photoactivation is delayed for five minutes after the manipulation of the resin cements, which is clinically feasible. Shrinkage stress was higher with immediate photoactivation for all resin cements and resulted in higher shrinkage stresses mainly in the coronal third where debonding between resin cement and root dentin account for most failures.

CONCLUSION

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

1. The push-out strength of fiber posts to root dentin, E, and VHN of the resin cements were affected by photoactivation timing, resin cement type, and the root canal region.
2. Bond strength increased gradually with delayed photoactivation timing. Significantly higher push-

Table 8: *Mean (SD) of Elastic Modulus in Gigapascals (GPa) for Each Resin Cement (n=5) (ext.)*

Root Third	RelyX ARC			
	Immediate	3 Min	5 Min	Pooled Average
Coronal	8.6 (0.7)	8.4 (0.4)	8.9 (0.1)	8.6 (0.4) A
Middle	6.7 (1.8)	6.9 (0.9)	7.2 (1.0)	6.9 (1.2) B
Apical	4.9 (1.1)	5.5 (0.3)	5.8 (0.8)	5.4 (0.8) C
Pooled average	The mode of activation was not significant (p=0.701).			

out strength values were obtained for RelyX Unicem with a five-minute delayed photoactivation timing.

3. The higher push-out strength of the fiber post is achieved on the coronal root region. The bond strengths decreased significantly in the apical region.
4. The residual shrinkage stress decreased with the three- and five-minute delayed photoactivation timings compared with the immediate activation timing.

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Regulatory Statement

The primary institution for this manuscript was the Federal University of Uberlandia.

Conflict of Interest

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

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